

For Reference

NOT TO BE TAKEN FROM THIS ROOM

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex LIBRIS
UNIVERSITATIS
ALBERTAENSIS





Digitized by the Internet Archive
in 2019 with funding from
University of Alberta Libraries

<https://archive.org/details/ASmith1967>

THE UNIVERSITY OF ALBERTA

BEHAVIOUR OF CERTAIN CULTIVATED GRASSES
IN RESPONSE TO INDUCED CHANGES IN THE ENVIRONMENT

FACULTY OF GRADUATE STUDIES
DEPARTMENT OF PLANT SCIENCE
DIVISION OF HORTICULTURE

by

AUSTIN L. SMITH

EDMONTON, ALBERTA

MAY, 1967

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Behaviour of Certain Cultivated Grasses in Response to Induced Changes in the Environment" submitted by Austin L. Smith, in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

In experiments with five varieties of creeping bentgrass Agrostis palustris Huds., significant behavioural patterns were observed both in response to low temperature preconditioning and to the effect of subsequent photoperiod. While it was apparent that low temperature preconditioning was an important factor in inducing creeping bentgrass to flower, one cultivar 'Toronto' did not respond to the same extent as the others. This grass was also more vigorously vegetative, producing greater numbers of stolons under conditions that were imposed.

The growth regulator CCC showed a significant effect in retarding growth of the intercalary meristems of creeping bentgrass. Treatment with this chemical resulted in plants that had significantly shorter stolons but the number of nodes produced was not affected. CCC appeared to have no effect in inducing Toronto creeping bentgrass to flower.

CCC proved to be effective in promoting rhizome initiation in Merion bluegrass but showed no measureable effect on growth.

During the early stages in the growth of creeping bentgrass, sucrose was found to be the major sugar accumulating in tissues, however a noticeable drop in the levels of this sugar was noted with the onset of stolonization. The appearance of the polysaccharide fructosan was associated with stolon production and there

was greater accumulation of this carbohydrate by the end of the experiment in plant materials that had received inductive temperature treatment.

TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION	1
LITERATURE REVIEW	
Characteristics of creeping bentgrass	3
The influence of temperature and daylength on the growth and flowering of grasses	6
Growth regulation by chemical means	12
Soluble Carbohydrates on cool-season grasses	17
MATERIALS AND METHODS	
A. Daylength and Temperature Studies	24
(1) The response of creeping bentgrass strains to photoperiod	24
(2) The effect of temperature and short-day pre- conditioning on creeping bentgrass 'Toronto' ...	26
(3) The effect of temperature preconditioning on the behaviour of creeping bentgrass	26
B. The Effects of CCC on Growth and Development of Turfgrasses	27
(1) On creeping bentgrass	27
(2) On Kentucky bluegrass	29
(i) Effect of CCC on dry matter yield	29
(ii) Effect of CCC on rhizome initiation	30
C. Soluble Carbohydrate Studies	32
(1) Harvesting of samples	37
(2) Extraction of samples	37
(3) Thin layer chromatographic separation	38
(4) Development and scanning	38
(5) Preparation of standard curves	29
RESULTS AND DISCUSSION	
A. Daylength and Temperature Studies	40
B. The Effect of CCC on Growth and Development of Turf- grasses	56
(1) On Toronto creeping bentgrass	56
(2) On Merion bluegrass	66
C. Soluble Carbohydrate Studies	70
SUMMARY AND CONCLUSIONS	75
LITERATURE CITED	78
ACKNOWLEDGEMENTS	84

LIST OF FIGURES

	<u>Page</u>
1. Plant container with glass panel used for rhizome initiation studies	31
2. (a) and (b). Stage of growth of Toronto creeping bentgrass taken from the field (chilled) and greenhouse (unchilled) after 18 days in the growth chamber	34
3. (a) and (b). Stage of growth of Toronto creeping bentgrass taken from the field (chilled) and greenhouse (unchilled) after 31 days in the growth chamber	35
4. (a) and (b). Stage of growth of Toronto creeping bentgrass taken from the field (chilled) and greenhouse (unchilled) after 50 days in the growth chamber	36
5. Effect of variation in photoperiod on growth of pre-chilled Congressional creeping bentgrass	44
6. Effect of variation in photoperiod on growth of pre-chilled Penncross creeping bentgrass	44
7. Effect of variation in photoperiod on growth of pre-chilled Waukanda creeping bentgrass	45
8. Effect of variation in photoperiod on growth of pre-chilled Northland creeping bentgrass	45
9. Effect of variation in photoperiod on growth of pre-chilled Toronto creeping bentgrass	46
10. Effect of 8 hour photoperiods on five strains of creeping bentgrass taken from the field on October 25th ...	47
11. Effect of 16 hour photoperiods on five strains of creeping bentgrass taken from the field on October 25th ...	47
12. Effect of inductive pretreatment (daylength + temperature) on greenhouse and field-grown Toronto creeping bentgrass	50
13. Effect of inductive pretreatment (daylength + temperature) on greenhouse and field-grown Toronto creeping bentgrass	50
14. Effect of interaction between daylength and inductive preconditioning on stolon length in Toronto Creeping Bentgrass	52
15. Effect of Variety on Stolon Production in Chilled and Unchilled Material	55

LIST OF FIGURES (Cont'd)

	<u>Page</u>
17. Effect of CCC on Toronto creeping bentgrass overwintered in the field and placed in growth chambers in early spring	58
18. Effect of CCC on Toronto creeping bentgrass overwintered in the greenhouse and placed in growth chambers in early spring	59
19. Differential effect of CCC on stolon length in chilled and unchilled Toronto creeping bentgrass	60
20. Effect of interaction of CCC treatment and preconditioning on number of nodes per stolon in Toronto creeping bentgrass	61
21. Effect of CCC on Toronto creeping bentgrass taken from the field at the end of the growing season	66
22. Effect of CCC on Toronto creeping bentgrass taken from the field at the end of the growing season	66
23. Standard curves for sucrose and fructose from Densitometer readings	72

LIST OF TABLES

	<u>Page</u>
I. Sampling Schedule for Soluble Carbohydrate Studies with Creeping Bentgrass 'Toronto'	33
II. The Effect of Field Exposure on Growth and Development of Creeping Bentgrass when Subsequently Exposed to Short and Long Days	41
III. The Inductive Effect of Short-Day/Low Temperature Pre-treatment on Creeping Bentgrass 'Toronto'	49
IV. The Response of Chilled and Unchilled Toronto and Penncross Creeping Bentgrass to Variation in Daylength	53
V. The Effect of the Growth Regulator CCC on Chilled and Unchilled Creeping Bentgrass 'Toronto'	57
VI. Effect of CCC on Field-grown Creeping Bentgrass 'Toronto' Placed in the Growth Chamber Following the Growing Season (October 15th)	63
VII. Effect of CCC on Growth of Merion Bluegrass Expressed as Dry Matter Yield of Clippings	67
VIII. Effect of CCC on Rhizome Initiation in Merion Bluegrass	68
IX. Changes in the Levels of Soluble Carbohydrates in Chilled and Unchilled Creeping Bentgrass 'Toronto' During Exposure to Short and Long Daylengths	74

INTRODUCTION

Creeping bentgrass (Agrostis palustris Huds.) is widely adapted to the temperate regions of North America for fine quality sports turf, particularly putting green surfaces.

Fragmentary records of the history of creeping bent on this continent support the belief that it was introduced to the Eastern coast of North America from Europe in unselected lots known as the "South German mixed bents". A number of ecotypes obtained from older greens of German bent origin are presumably the result of natural selection on the highly variable original populations.

Distinctions between creeping bent and other bent grasses are often confused due to lack of uniformity in creeping types and intermixtures with native species. Not all authors recognize Hitchcock's (33) treatment of the genus particularly the granting of specific status to creeping bentgrass.

The investigations reported here deal with five strains or cultivars of A. palustris viz., 'Toronto', 'Northland', 'Waukanda', 'Congressional' and 'Pennecross'. The first four are selections which are propagated vegetatively, i.e., by stolons. The latter is a hybrid of three strains for which seed is available commercially. Seed of the strain 'Pennecross' was obtained locally. Stolons of 'Toronto' and 'Waukanda' were obtained from the Milwaukee Sewerage Commission, Milwaukee, Wisconsin. Stolon material of 'Congressional' was obtained from Iowa State College, Ames, Iowa,

and material of the cultivar 'Northland' was received from Toro Manufacturing Company, Minneapolis, Minnesota.

From observations of these cultivars growing side by side in the field, it appeared that stolon growth was affected by length of day (photoperiod). In order to obtain information on the nature and extent of this, both on heading and stolonization in creeping bentgrass, investigations were undertaken using the controlled environments of growth chambers. Since prior exposure to low temperatures has been known to influence the response to photoperiod, it was necessary in later trials to include a study of the effect of this variable as well.

Because of variations in the growth habit of selected strains of creeping bentgrass, experiments were undertaken to determine differences in the responses of individual cultivars to seasonal changes. With a more precise knowledge of these responses it would then seem possible to formulate more specific management practises relative to certain types of growth habit.

Regulation of the growth of certain turfgrasses by chemical means also forms a part of the work reported. The effect of the growth retardant CCC on broad leaved plants is well known. In these studies, the effects of CCC were investigated on the initiation and development of seedheads as well as on the development of vegetative structures of grasses when exposed to inductive and non-inductive conditions.

LITERATURE REVIEW

Characteristics of Creeping Bentgrass

A. palustris is distinguished from other species of bentgrass chiefly by the long stolons, narrow stiff appressed blades, and the condensed (sometimes somewhat narrow) panicle (33). It is perennial, low and trailing or semi-trailing. Rhizomes may be present in some forms.

As a turf species, creeping bent is unexcelled as a fine-textured grass for putting green surfaces, being well adapted to close cutting and heavy use. Creeping bent spreads by stolons which root readily at the nodes. Tillers are also abundant and these contribute to a tightly knit sod. Dense growth on playing surfaces creates a heavy thatch which requires periodic thinning.

Arber (3) describes the barren shoots of A. palustris as attaining lengths up to two meters. Under swampy, natural conditions there is apparently an indefinite capacity for shoot elongation. The extravaginal branching of prostrate axes where the leaf sheath of the prostrate stem is burst open at the base by an axillary branch is also known in A. palustris (3). In fact Arber, in describing British forms of A. palustris, states that axillary branching is often a conspicuous feature although this is not the experience of the writer. Growth abnormalities such as the "mop habit" and proliferating inflorescences have also been observed in creeping bentgrass (3). 'Mops' are due to the capacity of one or more nodes to produce several new shoots at

certain stages of development. Occasionally a proliferating inflorescence is formed as the result of a vegetative shoot being borne laterally on a dry, shrivelled inflorescence axis.

Despite their common origin, selected strains of A. palustris show wide variation in growth habit, particularly the tendency to grain, swirl or thatch. 'Congressional' is highly rated for its dark green foliage and uniform growth habit. The 'Toronto' strain is noted for its vigorous growth habit especially during cool weather. 'Penncross', a seeded variety, is considered to be more disease resistant than some of the vegetative strains (56).

Holt and Davis (34) studied the differential response to fertilizer of the 'Arlington' and 'Norbeck' strains. They concluded that response to calcium deficiency is a reliable character for differentiating between the two.

Albrecht (1) investigated differences with regard to 2,4-D tolerance. He found that creeping bentgrass strains varied widely in tolerance to 2,4-D. 'Congressional' was less severely injured than was 'Toronto' when rated two, four and six weeks after spraying.

Differences in flowering and stolon habit in the 'Metropolitan' and 'Washington' strains have been reported by Allard and Evans (2). 'Metropolitan' failed to head in five months when placed in photoperiods of ten to eighteen hours after overwintering in the field at Arlington, Virginia. The strain 'Washington' from the same source, flowered only in the sixteen and eighteen hour regimes. Both strains required a minimum daylength of thirteen hours for stolon growth to occur.

Seed yields in different species and varieties of bentgrass were studied by North and Odland (52). They found that the 'Metropolitan' and 'Virginia' strains of A. palustris possessed higher seed-yielding ability than the 'Washington' variety. On the Eastern U.S. coast much of the bentgrass harvested for seed at that time (1934) was found in practically pure naturalized stands (52).

Juska (38) reported on the shade tolerance of eleven bentgrass varieties and concluded that those which performed best in sunlight were also better under shade.

Holt and Payne (35) studied variation in growth and other characteristics of open-pollinated seedling progenies of forty-nine strains of creeping bent. They cited earlier work by De France in which the latter observed that seed from selfed plants indicated considerable heterozygosity in the parent plant of a particular line. In their investigations, Holt and Payne noted significant differences between, and even within strains for total growth, texture, density, drought tolerance and type of growth. Stuckey and Banfield (64) also found widely diverging morphological types with varying chromosome numbers in studying open-pollinated progenies of Agrostis tenuis.

Inherited differences in ability to withstand low winter temperatures have been noted in bentgrass strains (60). Beard (6) found the more homogeneous vegetative strains of creeping bentgrass, 'Toronto' and 'Cohansey', to be more tolerant of extended ice cover than the heterogeneous seeded type 'Seaside'. 'Toronto', which is

known to be adapted to the more northern grass growing regions, withstood ice cover better than the 'Washington' strain.

The Influence of Temperature and Daylength on the Growth and Flowering of Grasses

Garner and Allard (27) were the first to advance the concept of photoperiodism, using flowering in angiosperms as the basis of response. The influence of length of day, or more correctly night, on plants, is known to be modified or counteracted by many environmental factors particularly temperature. For example, Peterson and Loomis (54) found that floral induction in Poa pratensis L. required both low temperature and short daylengths, but initiation and development of flowers needed long days and moderately warm temperatures. Temperature may manifest its effect before, during or after the presence of the photoperiodic stimulus. Conversely, an unfavorable daylength before or during chilling treatment is known to influence flower induction in vernalizable species of grass (26, 54).

The complexity of the interrelation between vernalization and photoperiodic requirements, although the two phenomena may work independently of one another, is noted by Chouard (13). All possible combinations from obligate or partial photoperiodic and low temperature induction to none (neutral) can be found among various species of plants. Varietal or strain differences make it necessary to classify some species in more than one combination.

Although the flowering response is most frequently described in studies on vernalization and photoperiodism, other morphological changes occurring in temperate grasses have been attributed to these phenomena. The growth of rhizomes in Kentucky bluegrass, Poa pratensis L., was found to be associated with long days (24). The opposite was true of Canada bluegrass, P. compressa L. In both species upright growth habit was attributed to long days (18 hours), whereas the shoots grew in decumbent or semi-decumbent positions under photoperiods of 8.5 hours daily, regardless of temperature. Watkins (69), working with brome grass, Bromus inermis Leyss., observed behaviour similar to that for Kentucky bluegrass. In addition, he found the total number of shoots per plant to be greater under short days than under long days. Both the vegetative and reproductive responses appear to be controlled by daylength, i.e., long days are required for each response. The control of stolon formation in some bentgrasses by long days has also been reported by Smith (60).

Cooper and Calder (20) have proposed the following developmental sequence for flowering in temperate grasses: (a) the necessity for a juvenile phase in some species during which no induction can occur, (b) inductive response to cold and/or short days (or dark exposure) in which a plant attains "ripeness to flower", (c) floral initiation which may or may not be sensitive to photoperiod, and (d) subsequent development of the inflorescence. Within this sequence the specific requirements for light and temperature

at each stage vary considerably with species and variety and is related to climatic and geographic origin (17). Cooper (15) was able to correlate the climate or latitude of origin of populations of Lolium, Phleum, and Triticum with response to low temperature (15). He also observed that response to vernalization treatments by Lolium is related to the duration of the life cycle. Annual strains head rapidly with little or no requirement for low temperature, whereas perennial strains have an obligate requirement. Response to low temperature is one important mechanism determining optimum life cycle.

In similar tests of a range of timothy strains (Phleum spp.) which were all long-day plants requiring no low temperature or short day induction, Cooper (16) observed another effect of temperature. At high temperature (55° - 65° F), inhibition of flowering varied with the strain and was related to the May temperatures in the region of origin. Canadian and American strains headed at this temperature, Scandinavian strains were inhibited, and British material was intermediate in response. A British diploid pasture strain was found to require a much lower temperature for heading and became stoloniferous in the field in Britain. In other research with outbreeding populations of Lolium from different ecological origins, Cooper (14), found the flowering responses in the progeny to be associated with the natural photoperiods and temperature of the parent race's place of origin. He observed further that heading is uniform in a population

selected under these conditions, but that changing the photoperiod and temperature conditions revealed considerable genetic variability. The heading responses in Lolium as cited in the foregoing, appear to be typical of adaptive characters from phenotypically similar populations stabilized by selection, but based on differing genotypes whether in inbreeding or outbreeding populations.

Vernalization of plants at different stages of growth has been demonstrated in Festucoid grasses (20, 23). In some species the seed or seedling can be fully vernalized, while in others such as Phalaris tuberosa L., low temperature treatment is effective only after several leaves have expanded (40). From the results on Phalaris and those of Calder on Dactylis glomerata L. (10), the existence of a juvenile stage (20) is obvious in some temperate grass species. Evans (23) states that the need for some vegetative growth as a requirement for vernalization may be widespread among perennial temperate grasses. Cooper (20) believes that the juvenile phase in perennial grasses serves the function of preserving the perennial habit. Since perenniality is based on the maintenance of an adequate number of vegetative tillers over winter, any development which encourages this, rather than heading in late summer, will be selectively advantageous.

Perennial grasses which require vernalization must be revernalized each year (23). Where vernalization is incomplete, intervening high temperatures ($>15^{\circ}$ C) can result in devernalization, but vernalization treatment need not be continuous provided devernalizing temperatures do not intervene. Once vernalization is complete,

devernalization can not occur. The process of vernalization occurs in the shoot apex whether in intact plants or embryos. The requirement of annual reversionalization implies a non-transmissible stimulus in induced apices since there is no dilution of effect through growth, or devernalization of fully vernalized apices (23). Thus vernalized apices can not induce later-formed neighboring apices. Evans (23) suggests that the role of vernalization may be more to prevent floral initiation in the fall since ear initiation on a tiller puts an end to further leaf production that season.

In many long-day Festucoid grasses, Poa pratensis, Bromus inermis, Lolium perenne, and Agrostis tenuis, which are vernalizable by low temperatures, short day vernalization is also known (19, 20, 54). It also occurs in the Festucoid species Agrostis canina, A. alba, and A. stolonifera, which do not respond to low temperature.

Nearly all vernalizable species also require long days for floral initiation, and the nearer a plant is to being fully vernalized, the fewer long days are required for initiation (53).

Evans (23) believes that vernalization may in some way increase the receptivity of the shoot apex to substances translocated from the leaves in long days. It is unlikely that short-day induction is due to the same processes as cold induction since the leaf is the organ of perception in the former and the shoot apex in the latter.

From data collected on the temperature and daylength requirements for flowering in grasses, it can be seen that all species of the sub-family 'Festucoideae' are either long-day or day-neutral plants (23). In the tribe 'Agrostideae', Agrostis alba is reported by some workers to have no inductive requirement for low temperature, but other experimenters have shown it to have an obligate response to vernalization (23). A. nebulosa and A. palustris show no cold temperature response but are reported to be long-day with regard to photoperiod (20, 59). Cooper (20) found that A. alba, A. canina, A. stolonifera and A. tenuis all have an inductive requirement for short days but show little or no effect due to cold. Evans (23) states, however, that strains and races within a species may vary considerably and this variation should be borne in mind when considering flowering requirement classifications based on work with only one strain or clone. Furthermore, many of the classifications are based on work with a narrow range of temperatures and photoperiods.

Initiation, even though plants were previously induced by low temperature or short days, may be delayed or prevented entirely by exposure to an inappropriate photoperiod and/or temperature following induction. Daylength effect on flower initiation is often modified by temperature (23). In long-day species of temperate grasses, high night temperatures (12° - 18° C) often suppress floral initiation although blind shoots may be formed (16, 57).

In short-day grasses, on the other hand, high night temperatures may have the opposite effect (23).

The course of inflorescence development is also subject to the environmental influences of daylength and temperature. Evans (22) found that slow development of the inflorescence is the reason for the late flowering habit of 'S23' ryegrass. The rate of inflorescence development appears to increase with temperature beyond the optimum for vegetative growth (23). The extent of vernalization does not affect the rate of inflorescence development (22, 23) but increasing temperature, daylength and number of long days promote it. The effect of daylength on development appears to be less pronounced than on initiation however (23).

Growth Regulation by Chemical Means

The growth retardant (2-chloroethyl) trimethylammonium chloride, henceforth designated "CCC", is known to affect initiation of floral primordia in some species of plants and the effect of this chemical on stem elongation has been well documented.

Cathey and Stuart (12) working with both long and short-day plants, observed little effect of CCC on flower initiation induced by photoperiod and temperature. Only the flowering times of Chrysanthemum morifolium Ramat. and Cleome spinosa L. var. 'Pink Queen' were noticeably delayed.

Stuart (63) was one of the first to demonstrate the suppression by CCC of vegetative growth and the initiation of flower buds in Azalea spp. Azaleas are reported to be day-neutral although very long days have been reported to delay flower initiation in some cultivars and long days have been reported to favor shoot growth. In experiments with Chrysanthemum and Poinsettia, Euphorbia pulcherrima Willd., Lindstrom and Tolbert (44) noted that the number of days to flowering was not substantially altered by CCC treatment. The main effects were shorter, more compact plants with thicker stems. Joiner and Sheehan (37), however, working with Poinsettia, were able to control height and flowering time by the use of CCC just as well as by manipulating propagating dates and photoperiod. A longer period of growth was required for the CCC-treated plants. In similar tests on Gardenia jasminoides 'Veitchii', which fails to flower when given continuous short nights, Joiner and Poole (36) found CCC effective in promoting initiation. In CCC-treated plants, treatment advanced the flowering date and increased the number of flowers in long nights. Treatments before the start of short nights were more effective than treatments after initiation of flower buds.

Flower formation in some photoperiodic plants can be suppressed by applications of CCC. In Bryophyllum daigremontianum, which can be induced to flower when given long days followed by

short days, Zeevaart and Lang (72) found complete suppression of flower development by CCC. The chemical was applied via the roots at a level of at least 1 g. per plant during short-day treatment. Zeevaart (71) observed strong suppression by CCC of flower development in the short-day plant Pharbitis nil Chois, strain Violet. The concentration used was 250 mg CCC/l applied to the roots prior to inductive treatment. He also observed that flower formation of Pharbitis grown in long days, i.e., non-inductive photoperiods, was promoted by CCC in that flower buds were formed at lower nodes than in controls. In studies of the long-day rosette plant Samolus parviflorus under inductive conditions, Baldev and Lang (7) found CCC to inhibit flower formation and stem elongation.

Early experiments with CCC, a derivative of choline, found it to be mostly ineffective on grass species (11, 66). An exception was the retarding effect on 'Thatcher' wheat. Plants, observed by Tolbert (67), had shorter, thicker stems, darker green leaves, and produced more tillers. Flowering was only slightly delayed. The concentrations used were from 10^{-2} M to 10^{-6} M usually applied as a soil drench. Results with turfgrass species viz. Kentucky bluegrass (39, 66), creeping red fescue (66), and creeping bentgrass (25, 66), proved it to be largely ineffective. Norris (51), however, recorded morphological changes in Kentucky bluegrass and creeping red fescue in greenhouse experiments using 0, 2½, 5, 10 and 20 lbs./A CCC applied as a foliar spray. Height of the plants was reduced compared to controls, rhizome length was decreased, and tillering was increased at low dosages. High

dosages caused severe growth retardation in every respect and either prevented an increase in tillering or reduced it. Leaf numbers per apical meristem were not significantly affected nor was total dry matter yield, except in winter-grown Kentucky bluegrass which produced higher yields at the lower rates of CCC application. Norris concluded that species with long stems (e.g. cereals) or rhizomes (e.g. Kentucky bluegrass) are more affected by CCC than the bunch type grasses.

Stoddart (62) conducted experiments with CCC on S51 timothy (Phleum pratense L.). Treatments from 1 M to 0.01 M applied at the 3rd, 6th or 8th leaf stage resulted in shortened culm length at heading, increased total head length and greater number of heads per plant. The latter two were at a maximum after 1 M treatments applied at the 6th or 8th leaf stages. No data were obtained to show that the greater numbers of heads was due to increased tillering, although this has been reported above (51, 67). The emergence of the inflorescence was not significantly altered and it was only slightly delayed at high dosages.

Cabler (9), investigated the effects of CCC on Cynodon dactylon L. var. 'Tifgreen' grown under 20, 60, 80 and 100 percent full sunlight. This grass, which does not grow well in shade, produced a dense growth under all regimes when treated with CCC. Norris (51) found no significant interaction between four light intensities and CCC on Kentucky bluegrass. He suggested that light duration, or length of day, could be the significant factor in growth discrepancies

during winter and summer experiments with this species.

Differences in effectiveness of CCC on production of underground parts of plants have been reported. Increased root to shoot ratios have been reported in Kentucky bluegrass, creeping red fescue, brome grass and wheat by Norris (51), and in bentgrass by Fletcher and Renney (25). Cathey and Stuart (12) studied the effects of three growth retardants on the rooting of *Chrysanthemum* cuttings. CCC was found to be inactive in these trials. Libbert and Urban (43), however, observed a significant increase in adventitious root production of Convolvulus sepium cuttings treated for 42 hours in 10^{-4} M CCC. Many twining plants of the Convolvulaceae are known to contain large amounts of gibberellin-like substances which inhibit root production. Libbert and Urban suggest that the increased rooting is due to treatment with the "anti-gibberellin" CCC.

Guttridge (30), applied CCC to strawberry plants and observed decreased petiole length, and suppressed initiation and length of runners. After an initial phase in which the responses were observed, the plants recovered and grew even more vigorously.

Stoddart treated Lolium temulentum L., a summer-annual grass, with CCC to observe the effects on soluble carbohydrates. Concentrations of from 0.01 - 0.05 M inhibited growth and increased the free sugar content of the plants. Concentrations of 0.05 M and higher, induced accumulations of large amounts of fructosan. The plants were grown under 18 hour days. Accumulation was especially noticeable under conditions of nitrogen stress and applications of nitrogen lowered the free sugar content.

Soluble Carbohydrates in Cool-Season Grasses

Sugars and sugar polymers in plants are referred to as reserve or storage carbohydrates. Bernatowicz, as cited by May (47), objects to the term "reserve carbohydrates" as having a teleological connotation and suggests "accumulate" as a better word. Because it is well-established, however, the use of the term "reserve" will be continued in this study. Weinmann (70), defines reserve substances as organic materials produced by the plant and stored in the more permanent organs at certain times, to be utilized later as a source of energy or as building materials.

For the synthesis of new materials plants require a source of energy and carbon skeletons and a supply of available nitrogen in addition to other mineral elements. The potential energy and carbon skeletons in the form of organic acids are supplied by the soluble sugars or reserve carbohydrates. The nitrogen and other mineral elements are supplied by the soil solution.

McIlvanie, as cited by Troughton (68), calculated the ratio of percentage reserve carbohydrates to percentage total nitrogen (C/N) in roots and shoots of grasses during the growing season. He noted a low ratio during maximum vegetative growth, i.e., carbohydrates being utilized more rapidly than nitrogen stores, and a higher ratio at the beginning and end of the growing season. Weinmann, et. al., as cited by Troughton (68), however, were unable to detect such pronounced trends. The general situation for all plants has been summarized by Steele (61), who states that the amount of ammonium- or nitrate-ions absorbed by a plant affects the carbon/nitrogen ratio in its tissues.

A low ratio leads to vigorous vegetative growth, while a high ratio encourages development of reproductive organs.

The principal carbohydrate reserves in grasses are sugars, fructosans and starch (47). According to Weinmann and Reinhold, as cited by May (47), the fructosan-containing grasses are considered to be the cool-season, temperate species. Grasses accumulating sugars and starch are essentially those adapted to warmer climates.

From the literature it would appear that some confusion exists in carbohydrate terminology. The term 'sugars' in this study refers to a class of carbohydrates comprising the mono-, di-, and oligosaccharides. 'Free sugars', as defined here, are the monosaccharides glucose and fructose, and the disaccharide sucrose (41). Oligosaccharides are compounds which vary in length from the two monomeric units of sucrose to the longer oligosaccharide classes such as 'fructans' and the raffinose family (5). Distinguishing the higher oligosaccharides from polysaccharides is often difficult because, as Axelrod (5) suggests, there is reason to believe that oligosaccharides are merely intermediates in the formation or breakdown of polysaccharides. Strictly speaking, the fructans are large enough to be considered polysaccharides but their occurrence with and structural similarity to the lower molecular weight oligosaccharides, warrants their consideration as a class (5). The polysaccharides of importance to these studies are the polyfructosans which are characterized by their insolubility in aqueous ethanol, solubility in water and sensitivity to hydrolyzing agents (50). Fructans and polyfructosans are both polymers

of fructose formed by the elongation of a sucrose molecule by the successive addition of fructose moieties in glucosidic linkage (5). They are both non-reducing compounds and differ in their degree of polymerization, molecular weight and solubility. The polyfructosans are best described as "reserve polysaccharides" and are hydrolyzed for metabolic use by hydrolytic enzymes (5). There is evidence that these polymers of fructose are synthesized from sucrose through enzymic transfructosidation (5, 29, 31).

In the grasses, fructose polymers occur in all parts of the plant - roots, stems, and leaves (31). Waite and Boyd, cited by Hansen, et. al. (31), noted however, that stems of plants usually have much higher levels of fructose polymers than do leaves of the same plant. This is in agreement with the findings of Baker, and Sprague and Sullivan as reviewed by Green (29). The amounts found in any one part of the plant have been definitely related to season, clipping or grazing, and the influence of fertilizers (31, 70); the accumulation of reserves being dependent on such factors as nutrient supply, water supply and temperature (47).

Fructose polymers have been isolated from several grasses including the creeping bents (31). Jordan, according to Green (29) found fructan to be the best indicator of reserve carbohydrate in creeping bentgrass. Free fructose, and fructose from sucrose, were of least importance.

Seasonal trends in soluble carbohydrates have been traced by Lopatecki (46), McIlvanie, as cited by Troughton (68), Archbold (4), and others (68). McIlvanie found maximum reducing sugars with

rapid vegetative growth, maximum sucrose with differentiation, and greatest reserve polysaccharides with the rest period prior to secondary herbage growth or the production of new shoots in autumn. Archbold observed sucrose to be the predominant sugar in leaves of barley, the content falling with stem elongation and ear development. Fructosan was greatest in stems and accumulated as growth was completed. Lopatecki, working with wheat, noted the presence of glucose, fructose, sucrose, oligosaccharide and fructosan in the stems. Oligosaccharides were predominant prior to ripening of the grain. Fructosans accumulated after the build-up of sucrose which increased during the milk stage when monosaccharides were declining. The build up of reserves is most noticeable in autumn; at this time of year carbohydrates accumulate in the roots of perennial grasses (68, 70).

In studies with cool-season turfgrasses, Green (29), found oligosaccharides other than sucrose to be the best indicators of carbohydrate reserves in leaves following nitrogen fertilization. Leaf tissue from plants growing in the greenhouse environment was considerably lower in oligosaccharide than field-grown material. Fructosan levels in leaf tissue were consistently low throughout. The mono- and disaccharides did not show differential levels attributable to nitrogen treatments and Green asserts that since they are active metabolically they do not form part of the typical carbohydrate reserve. He concludes, however, that due to the parallel behaviour between concentrations of free sugars and sugar polymers during rapid growth and again during rest periods,

the free sugars represent an important component of the total carbohydrate reserve.

Sullivan and Sprague (65) noted a similar regular loss of fructosan from both the stubble and roots of perennial ryegrass with increase in temperature. In creeping bentgrass, maximum loss of reserve carbohydrates associated with optimum growth was at 70° F according to Jordan (Green, 29). Weinmann (70) concluded that seasonal trends in individual carbohydrate fractions vary for different grass species and environmental conditions and believes no generalizations can be made.

Accumulation of sugars in tissues above that needed for immediate growth can result in anthocyanin production in young seedlings according to Goodwin (28). The pigment is often formed transiently in the leaves of young seedlings particularly when carbohydrate metabolism has been disturbed by low temperatures or nitrogen deficiency (32). In the fall-coloring of leaves of some species of plants, anthocyanin production is dependent on climatic factors for full development. It is assumed that this fall color is associated with the accumulation of sugar from starch degradation in dying leaves but this has not been proven (28).

The anthocyanins are widely distributed in higher plants (32). They have been more widely studied from a genetic and taxonomic viewpoint rather than from a biochemical one, hence the biological reactions underlying their synthesis and function are somewhat obscure.

Anthocyanins occur in most plant organs - particularly in leaves, stems and seeds of grasses. In many plants, production, concentration and distribution of anthocyanins have been found to be under genetic control (Scott-Moncrieff, Dodds and Long, Lawrence as cited by Harborne, 32). Aside from their contribution to flower, fruit and leaf color, few undisputed functions for anthocyanins have been put forward.

Very little is known regarding the biosynthesis of anthocyanins although it is known that they are related indirectly with carbohydrate metabolism (32). D-glucose, among others, is one monosaccharide which has been shown to be attached to pigments by glycosidic linkage (32). Siegelman and Henricks as cited by Borthwick and Hendricks (8), have shown anthocyanin synthesis to be controlled by a photoreaction having a maximum absorption in the red part of the spectrum. As opposed to the low energy reversible red-far-red reaction, however, this is a high energy reaction which arises from continued excitation of the two forms of the photo receptive pigment.

In experiments with CCC, reviewed earlier (62, 71, 72), the occurrence of anthocyanin in treated plants appeared to be a characteristic side-effect. Zeevaart and Lang (72) noticed that CCC applications resulted in increased anthocyanin production in the leaves of Brvophyllum daigremontianum. In Pharbitis nil, Zeevaart (71) observed that control plants contained more anthocyanin in their stems than did CCC-treated ones. Stoddart's (62) experiments with timothy resulted in anthocyanin banding of leaves at the higher

concentrations of CCC applied.

MATERIALS AND METHODS

A. Daylength and Temperature Studies

Preliminary work done in the greenhouse with the creeping bentgrass variety 'Congressional', indicated that the strain was sensitive to changes in photoperiod. Test material, when exposed to long (18 hour) photoperiods, gave extensive stolon production whereas on exposure to photoperiods of 8 hours duration, short, leafy shoots were produced.

While one might expect all strains of A. palustris to respond to environment in similar fashion, the variety 'Toronto' reacted to daylength in a manner quite different to that of three other varieties growing beside it.

As a result of these observations it was decided to investigate the effect of photoperiod on four creeping bentgrass clones and one seeded cultivar, and further to determine whether or not this response had been pre-conditioned by exposure to the low temperatures of winter.

(1) The Response of Creeping Bentgrass Strains to Photoperiod

An experiment was conducted early in the spring to determine the effect of various photoperiods on grasses which had been wintered in the field. Four clones of A. palustris viz., 'Congressional', 'Northland', 'Toronto' and 'Waukanda' as well as the seeded type 'Penncross' were selected for exposure to the controlled environment

of the growth chamber. Later in the year, following a summer's growth in the field, the experiment was repeated in order to compare the behaviour of these grasses to photoperiod presumably after the chilling effect of winter had worn off.

In both cases, $3\frac{1}{2}$ " plugs were taken from the field using a turf core sampler and grasses were maintained in the clipped condition prior to use. Each plug was divided into several sections and uniform portions were placed in 4" plastic pots in a 3 soil : 1 sand : 1 peatmoss mixture containing added nutrients. Before being placed in the growth chambers, the grasses were allowed to grow in the greenhouse under natural daylength for one week.

Eight pots of each variety were soaked until moist, then moved into the growth chambers set to give 8 and 16 hour photoperiods. Temperature was held at 22° C during the light period and at 13° C during the dark. Light intensity at the pot rim was maintained at 1200 foot candles.

Observations on the material treated in the spring were made over a 6 week period and over an 11 week period for that taken in early fall. At the end of these experiments measurements of stolon length were made, and the numbers of nodes and seedheads were recorded. The five longest stolons from each pot were selected for measurement and node counts. Total seedhead counts were made on each pot.

Selected stolons were excised as closely as possible to the crown of the plant. Measurements were made from the cut end to the base of the bud shoot. The number of nodes was determined by counting

from the base of the stolon to the last node below the bud shoot. This procedure did not include the bud shoot node which was not sufficiently developed to be counted.

(2) The Effect of Temperature and Short-day Preconditioning on Creeping Bentgrass 'Toronto'

In work with Agrostis spp., A. alba, A. canina, A. stolonifera and A. tenuis were found to have an inductive requirement for short days but little or no requirement for cold (20).

In order to test the effect of short-day preconditioning on A. palustris, plugs of the strain 'Toronto' were overwintered in the greenhouse in 8 hour photoperiods. In the spring, the behaviour of this material was compared with that of grass overwintered in the field when both were exposed to 8, 12 and 16 hour photoperiods. Ten pots of each grass were soaked overnight and placed in three growth chambers. Light and temperature conditions were described in the foregoing experiment.

Measurements of stolon length and counts of seedheads and nodes were taken after 6 weeks as described earlier.

(3) The Effect of Temperature Preconditioning on the Behaviour of Creeping Bentgrass

Among the strains of creeping bentgrass tested it had become apparent that two general types were emerging; those that tended to maintain vigorous vegetative growth over a wide range of photoperiods, and those that could be more easily induced to flower.

In order to determine whether or not these responses might be preconditioned in each case by exposure to the chilling temperatures of winter, an experiment was undertaken in early spring to compare the behaviour of material that had been overwintered in the field with that of material that had been overwintered in the greenhouse.

The 'Penncross' strain, a heading type, was selected for comparison with 'Toronto', a strain noted for its vigorous vegetative habit.

Plugs of 'Penncross' and 'Toronto' were taken in the fall and overwintered in the greenhouse for comparison with material which had been overwintered in the field. No attempt was made to control photoperiod during overwintering.

Five pots of each strain, from both field and greenhouse material, were placed in 8, 16 and 24 hour photoperiods in the controlled environment of the growth chamber in early May. After six weeks, data were collected and recorded as in earlier experiments.

B. The Effects of CCC (2-chloroethyl) trimethylammonium chloride on Growth and Development of Turfgrasses

(1) On Creeping Bentgrass

Preliminary work with the 'Congressional' strain of creeping bentgrass indicated that a concentration of 800 ppm. CCC, applied as an aqueous soil drench, had a strong retarding effect on stolon growth, even when the grass was exposed to long days (18 hours).

In view of this, it was decided to follow up the effect of this chemical growth regulator on 'Toronto' material that had been pre-conditioned by low temperature exposure.

Plant material was taken from the field in the spring and potted as described earlier, except that all soil was removed from the roots of the plants. This was done to ensure uniform distribution of the chemical in the growing medium. The grass was clipped at potting time and moved to the greenhouse for two weeks prior to treatment. After two weeks all samples were again clipped to pot rim height and treated with 800 ppm. CCC applied at the rate of 100 ml. aqueous solution per pot. The formulation used was "Cycocel", 65 percent active dry formulation, manufactured by the American Cyanamid Company of Princeton, N.J. Ten pots including untreated controls were then placed in 8, 12 and 16 hour photoperiods. Data on heading, stolon length and number of nodes were collected at the end of six weeks.

Because of the apparent inability of unchilled 'Toronto' bentgrass to head in short or long photoperiods in most instances, an experiment was performed to see if the combined effects of CCC and long photoperiods would substitute for low temperature. In this experiment, photoperiods of 20 and 24 hours were used.

Field grown material was selected on October 15, 1965, potted, and twenty pots each were placed in the two daylength regimes. Half of the material was then treated with 800 ppm CCC as a soil drench. Light intensity in the cabinets was 1800 fc. at pot level.

Eleven weeks after treatment, the grasses in all pots were examined and measurements made according to the procedure adopted earlier..

(2) On Kentucky Bluegrass

Increased dry matter of clippings in Kentucky bluegrass due to treatment with CCC has been reported by Norris (51). He found the response to be associated with increased tillering at low dosages but also reported that the effect of CCC on rhizomes showed up as decreased growth which he recorded as rhizome length per plant. In Norris' work, no observations were made on numbers of rhizomes. One might expect, however, increased dry matter of clippings to be intimately connected with increased rhizome initiation.

Two experiments were undertaken - the first to check the effect of CCC on dry matter produced by leaf clippings of Merion bluegrass, and the second to see whether or not the chemical had any effect on rhizome initiation.

(i) Effect of CCC on Dry Matter Yield

Plugs of 'Merion' bluegrass were taken from the field, potted and allowed to remain in the greenhouse for two weeks prior to treatment. After two weeks the grass was clipped to 2 inches above the pot rim using a cardboard collar device to maintain a uniform height of cut. Twenty-five pots, or 5 pots per treatment, were sprayed with 0, 5, 10, 20 and 40 lbs/A CCC. A custom-made cabinet sprayer with an electrically operated overhead boom was

used for spraying. The spray boom was equipped with "Tee Jet" (No. 650067) fan-type nozzles and was calibrated to deliver 33 gals/A at a pressure of 40 p.s.i. The boom height was set at 18" above the pots. Special cardboard collars were made to prevent the spray from contacting the soil around the pot edge.

The grass in all pots was clipped after 2, 4 and 7 weeks and the clippings dried in an oven at 66° C for 48 hours. When cool, the clippings were weighed to the nearest 0.1 g.

(ii) Effect of CCC on Rhizome Initiation

In this experiment, special plywood plant containers (6" x 6" x 10") were constructed, (see Fig. 1). One side of the container was fitted with a glass panel so that following treatment with CCC, the box could be laid horizontally with the glass side at the top. This theoretically would place a geotropic and later a phototropic stimulus on rhizomes initiated subsequent to treatment and thus serve to distinguish these from structures initiated earlier.

Plugs of the test material were taken in June from established plots and the soil washed off the roots before potting since CCC treatments in this trial were to be applied as a dry soil mix. During washing, rhizomes from $\frac{1}{2}$ " to $1\frac{1}{2}$ " long were noted. The growing medium was three parts black loam to one part sand by volume. Peatmoss was not used in the medium because it was felt it might affect uniform mixing of the chemical. CCC was applied as a soil mix, using "Cycocel", 65 percent active ingredient, at the rates of



Fig. 1. Plant container with glass panel used for rhizome initiation studies.

0, 20 and 30 gms/cu. ft. After soaking, and clipping the grass to 2", fifteen containers or 5 per treatment were left in the greenhouse under natural light for 9 weeks. Maximum temperatures of 27° C during the day were recorded but temperatures were maintained as closely as possible to 13° C at night.

At the end of nine weeks, rhizome counts were made and the plants discarded.

The experiment was repeated in early fall except that 0, 10, 20 and 30 gms/cu. ft. of CCC were applied and rhizomes were counted after fourteen weeks. The day and night temperatures of the previous experiment were maintained and natural daylengths applied.

C. Soluble Carbohydrate Studies

Soluble carbohydrates in cool season grasses have been found to vary with management practise and changes in environmental conditions. In experiments reported here, morphological differences attributable to daylength and temperature treatment were noted in five strains of creeping bentgrass. Since it might be expected that changes in soluble carbohydrate levels are associated with these differences, it was decided to follow the behaviour of carbohydrate fractions during the course of the photoperiod work.

Experiments of a semi-quantitative nature were conducted in order to detect trends in specific carbohydrate fractions prior to the various changes in growth habit. Conditions in the growth chamber were the same as in earlier experiments.

The analytical technique used was based on a method employed by Green (29) in studies on cool-season turfgrasses.

Green's quantitative analysis employed densitometer readings from paper chromatograms.

In these studies, samples were harvested as total material above the pot rim which normally included both leaf and stem tissue. In samples harvested early in the growing period, a higher ratio of leaf to stem was observed. As plants grew and produced stolons, this ratio fell so that later samples contained a higher actual weight of stem to leaf tissue. This can be seen from Figs. 2, 3 and 4. Samples in triplicate, were harvested according to the following schedule:

TABLE I. Sampling Schedule for Soluble Carbohydrate
Studies with Creeping Bentgrass 'Toronto'

Grass	Source	Start of Experiment	Photoperiod	Sampling Date
Toronto 1	Field	February 15	8 hrs. and 16 hrs.	March 5, March 19, April 6
Toronto 2	Greenhouse	February 15	8 hrs. and 16 hrs.	March 5, March 19, April 6



(a) 8 Hours - Chilled = Cold; Unchilled = Warm



(b) 16 Hours - Chilled = Cold; Unchilled = Warm

Fig. 2 (a) and (b). Stage of growth of Toronto creeping bentgrass taken from the field (chilled) and greenhouse (unchilled) after 18 days in the growth chamber.



(a) 8 Hours - Chilled = Cold; Unchilled = Warm



(b) 16 Hours - Chilled = Cold; Unchilled = Warm

Fig. 3 (a) and (b). Stage of growth of Toronto creeping bentgrass taken from the field (chilled) and greenhouse (unchilled) after 31 days in the growth chamber.



(a) 8 Hours - Chilled = Cold; Unchilled = Warm



(b) 16 Hours - Chilled = Cold; Unchilled = Warm

Fig. 4 (a) and (b). Stage of growth of Toronto creeping bentgrass taken from the field (chilled) and greenhouse (unchilled) after 50 days in the growth chamber.

(1) Harvesting:

- (i) Samples (2 gms. fresh weight) were taken at the end of the dark period (8:00 a.m.).
- (ii) Material was denatured in 100 ml. boiling 80 percent ethanol for five minutes, then
- (iii) Cooled, covered and stored in refrigerator at -18°C until extracted.

(2) Extraction:

- (i) Sample was mascerated for one minute in Waring Blendor and rinsed with 80 percent ethanol.
- (ii) Alcohol fraction. Sample filtered through Whatman #1 filter paper in a Buchner funnel connected to a vacuum flask. Boiling, 80 percent ethanol, was added and fraction brought to 250 mls. This fraction contained mono-, di-, and oligosaccharides.
- (iii) Water fraction. The filter residue from alcohol fraction was extracted with 150 mls. boiling water over vacuum. This fraction contained the polysaccharides.
- (iv) A 167 ml. aliquot from the alcohol fraction was taken and flash evaporated at 40°C to 10 mls. This was repeated with a 100 ml. aliquot from the water fraction.
- (v) Residues from water and alcohol extraction were removed by repeated rinsings and brought to 10 ml. final volume in volumetric flasks.

(vi) Hydrolysis. Alcohol fraction was divided into two 5 ml. volumes. One portion was hydrolyzed by making it 1 N with concentrated HCl. This was repeated with the water fraction. These were then stored overnight at room temperature.

(3) Thin Layer Chromatographic Separation:

- (i) Eastman Chromogram Sheets (Silica gel Type K 301R2 without fluorescent indicator) were used in all separations.
- (ii) Water and alcohol extracts were filtered and spotted in triplicate using 5 λ volumes in each case.
- (iii) Chromatograms were developed in $3\frac{1}{2}$ hours using an n-propanol, benzyl alcohol, 85 percent formic acid and water (50/72/20/20 v/v) solvent.
- (iv) Sheets were removed and dried at room temperature overnight.

(4) Developing and Scanning:

- (i) Spots were developed using aniline- H_3PO_4 indicator (1 part 2 N aniline in n-butanol to 2 parts 2 N H_3PO_4 in n-butanol v/v).
- (ii) Chromatograms were then placed in an oven at 105°C for 12 - 15 minutes to develop color of the spots.
- (iii) Spots were observed under ultraviolet light and scanned with a Photovolt Electronic Densitometer Model 525. A metal aperture disc without filter was used to centre the spots. Measurements were made along the Density Scale of the recorder and these readings were compared with those prepared from known concentrations of standards.

(5) Standard Curves:

- (i) Standard curves were plotted from densitometer readings of thin layer chromatograms on which known concentrations of sucrose and fructose had been spotted.
- (ii) Density readings were plotted against concentrations to determine if the resulting curves formed a straight-line relationship.

RESULTS AND DISCUSSION

A. Daylength and Temperature Studies

From observations of five strains of A. palustris growing side by side in the field, variation in growth habit, particularly the tendency to produce stolons and seedheads was noted. Allard and Evans (2) reported differences in heading of the 'Washington' and 'Metropolitan' strains of A. palustris when exposed to various daylengths. In their studies on field-grown material, 'Metropolitan' failed to head in photoperiods of 10 to 18 hours and 'Washington' flowered only in the 16 and 18 hour regimes. Length of day affected stolon growth as well, and both strains required a minimum daylength of 13 hours for stolon production to occur.

The response to daylength of 4 creeping bentgrass clones and one seeded type 'Penncross', taken in early spring and fall, are shown in Table II. From these data it may be seen that long days (16 hours) appear to favour seedhead production when the grasses have previously been exposed to the low temperatures of winter. In comparing data in Table II, it should be noted that measurements of spring material were taken after 6 weeks in the growth chambers while data for the fall-grown grasses were taken after 11 weeks. Seedheads were observed, however, in the fall-grown material after 5 weeks in the growth chambers and there was no increase in the number produced after this time.

TABLE II. The Effect of Field Exposure on Growth and Development of Creeping Bentgrass when
Subsequently Exposed to Short and Long Days

Variety	Seedheads ¹		Stolon Length (cms)		Number Nodes ²		Internodes (cms)		Stolons ¹	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Congressional	8.3	0.0	11.8	0.0	5.7	0.0	2.1	0.0	0.0	0.0
Northland	0.0	0.0	24.1	22.5	9.3	10.2	2.6	2.2	40.0	40.0
Pennecross	3.3	0.0	13.2	0.0	5.9	0.0	2.2	0.0	0.0	0.0
Toronto	0.0	0.0	16.9	20.7	8.1	9.7	2.4	2.1	25.0	25.0
Waukanda	2.3	0.0	13.0	0.0	6.1	0.0	2.1	0.0	0.0	0.0
Congressional	46.0	3.5	27.1	32.7	8.1	15.1	3.3	2.1	51.0	51.0
Northland	30.5	2.5	34.7	32.1	10.9	16.7	3.2	1.9	81.0	81.0
Pennecross	31.3	14.7	26.0	27.1	8.1	15.4	3.2	1.7	61.0	61.0
Toronto	0.0	7.3	24.6	27.7	9.5	16.5	2.6	1.7	109.0	109.0
Waukanda	50.5	13.5	26.3	25.5	8.1	14.1	3.2	1.8	57.0	57.0
Means	17.2**	4.1					2.7**	1.3		

Data based on 4 pots (reps.).

¹Mean number per pot.

²Mean number per stolon; based on 5 longest stolons per pot.

It will be noted from the data in Table II for seedhead production that regardless of subsequent photoperiod treatment, the chilling effect of winter temperatures had a highly significant effect on developmental processes. This would indicate further that the chilling effect of winter temperatures is offset by warm temperatures of summer when the plants are growing in the field. Studies of the inductive effect of low temperatures have shown that perennial grasses requiring vernalization must be revernalized each year (23). It is interesting to note however, that the species A. palustris has been reported by Cooper and Calder (2) to show no cold temperature response although they found it to be a long-day plant with regard to photoperiod. The strain of A. palustris on which these observations are based is not reported however, and Evans (23) states that strains and races within a species may vary considerably. This variation should be taken into consideration when looking at flowering requirement data based on work with only one strain or clone.

In Table II, of the three strains from spring-grown material which produced seedheads in 8 hour photoperiods, 'Congressional' and 'Waukanda' had improperly developed heads. This suggests that although inflorescences were initiated, short days prevented further development. Even in 12 hour photoperiods some inflorescences in these two varieties showed heads which had not fully emerged from the sheath. This has been described in other grasses (22). Of the five strains of A. palustris tested, the production of seedheads in all but the

cultivar 'Toronto' appeared to be significantly increased in long days. The existence of some other factor operating in the developmental sequence in the case of the strain 'Toronto' seems likely.

Although long days resulted in increased heading in four of the five strains studied, the strain 'Toronto' failed to flower except where field-grown material was brought in during the fall and exposed to 16 hour days. An explanation of this apparent contradiction is difficult as the temperature and daylength requirements for heading in this strain appear to be rather complex. In subsequent experiments with the strain 'Toronto', heading was erratic and unreliable even though it was subjected to a wide range of treatment.

It also may be seen from the data in Table II that two general types of creeping bentgrass were emerging. The strains 'Toronto' and 'Northland' appeared to maintain a vigorous stolon growth over a wide range of photoperiods, whereas the cultivars 'Congressional', 'Penncross', and 'Waukanda' were apparently more easily induced to flower. These differences may be seen in Fig. 5, 6, 7, 8 and 9 of spring-grown material and in Figs. 10 and 11 from material taken in the fall.

While differences in stolon length between cultivars are apparent in 8 hour photoperiods, these differences tend to disappear in material growing in 16 hour daylengths. Also, there is very little difference between spring and fall-grown grasses in 16 hour photoperiods despite the fact that data in the fall trial were taken after a five-week longer growing period. Of interest

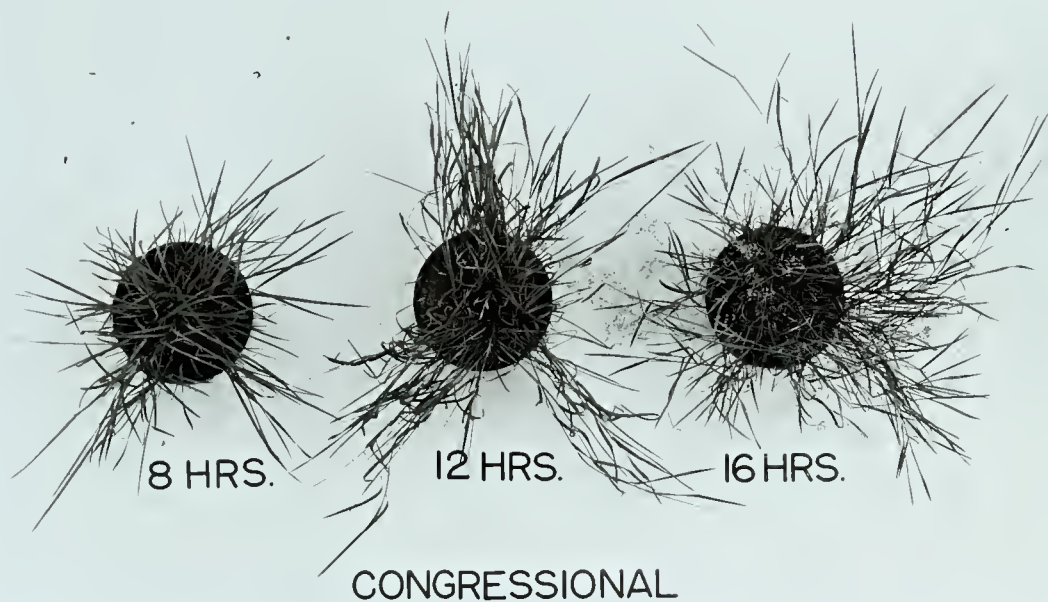


Fig. 5. Effect of variation in photoperiod on growth of prechilled Congressional creeping bentgrass.

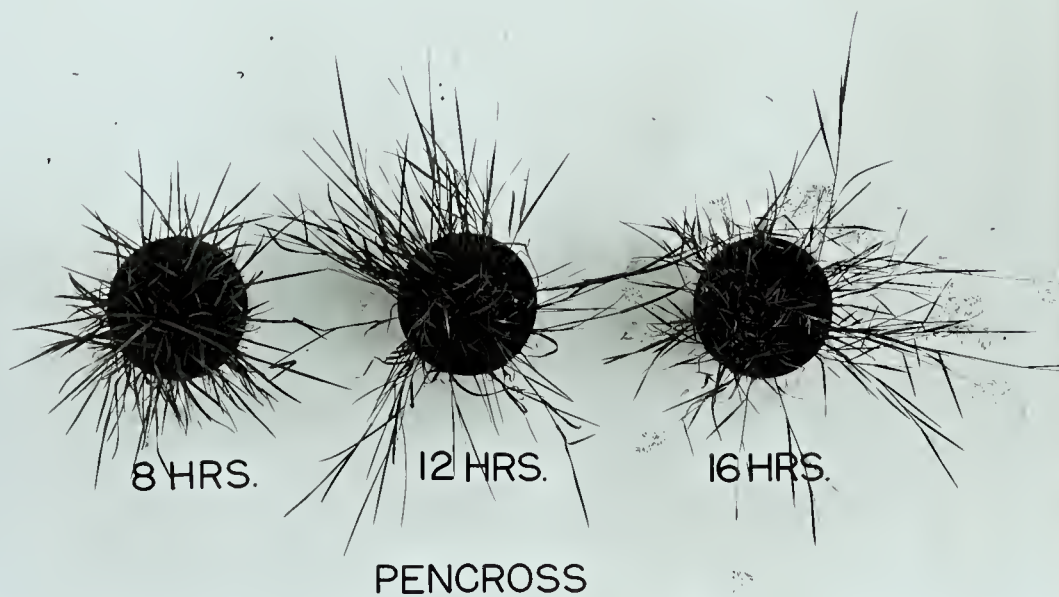


Fig. 6. Effect of variation in photoperiod on growth of prechilled Penncross creeping bentgrass.

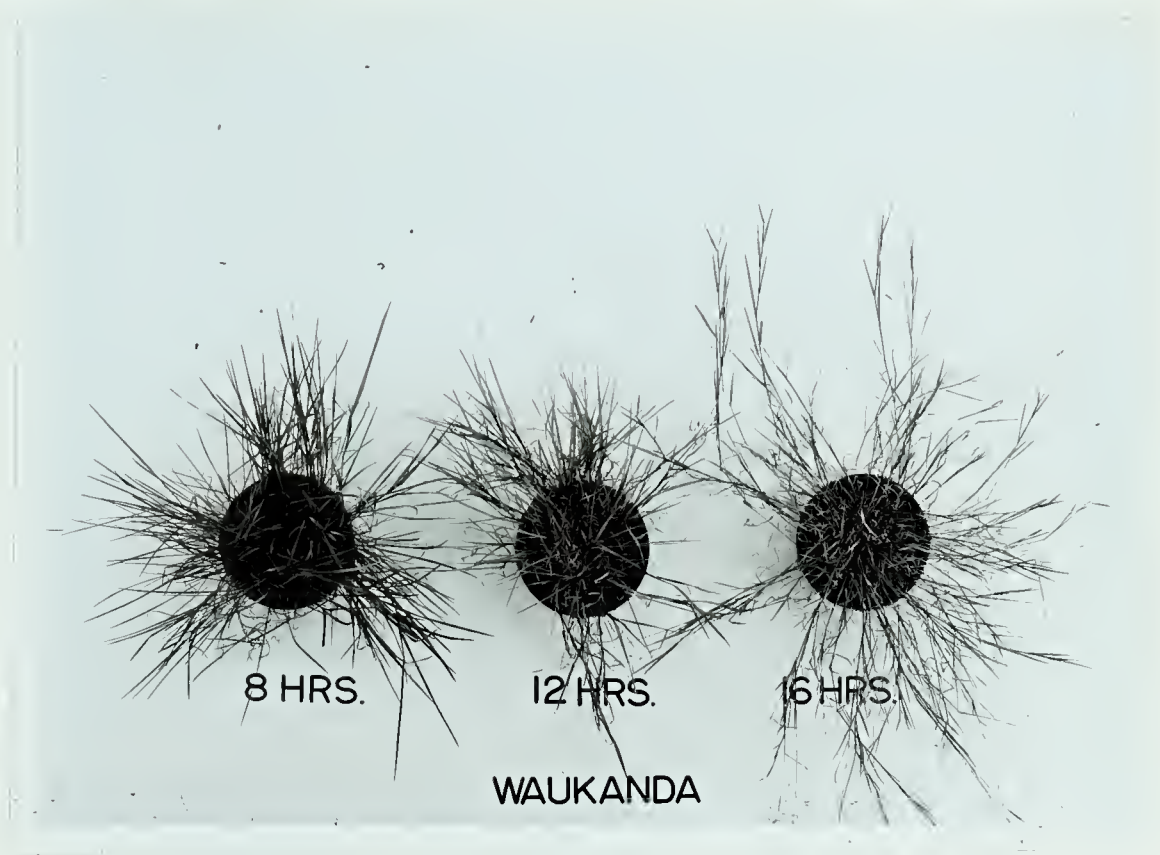


Fig. 7. Effect of variation in photoperiod on growth of prechilled Waukanda creeping bentgrass.



Fig. 8. Effect of variation in photoperiod on growth of prechilled Northland creeping bentgrass.

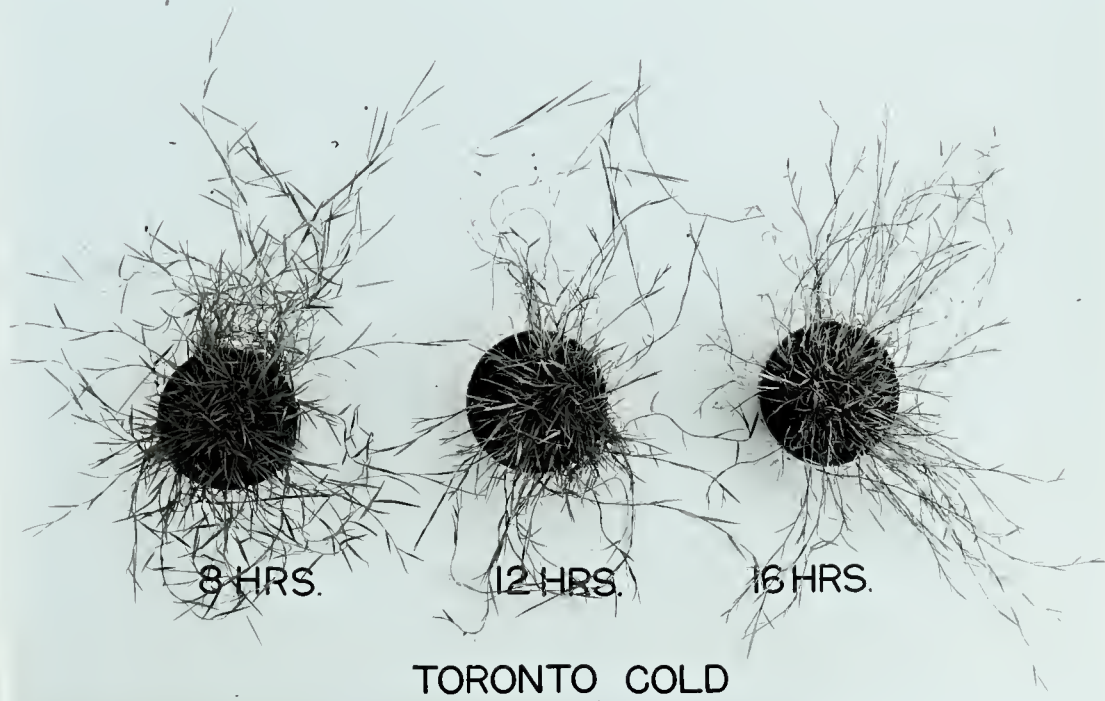


Fig. 9. Effect of variation in photoperiod on growth of prechilled Toronto creeping bentgrass.



Fig. 10. Effect of 8 hour photoperiods on five strains* of creeping bentgrass taken from the field on October 25th.



Fig. 11. Effect of 16 hour photoperiods on five strains* of creeping bentgrass taken from the field on October 25th.

*T₁ = Toronto
T₂ = Penncross
T₃ = Congressional

T₄ = Waukanda
T₅ = Northland

here is the consistently greater internode length in spring-grown material. Statistical analyses confirm that this effect is highly significant. The explanation for this difference lies in the fact that fall-grown grasses produced considerably more nodes per unit length of stolon. Although one might normally expect a difference in number of nodes as a result of the five week longer growing period for the fall material, the similarity in length of stolon within each variety is not accounted for.

Other morphological differences noted among the 5 strains were texture of stolons and degree of pigmentation. The variety 'Northland' produced the finest textured stolons. Anthocyanin production in internodal areas and leaf bases appeared to be greatest in the strain 'Waukanda' particularly in spring-grown material taken from 16 hour photoperiods.

In flowering classifications reported by Cooper and Calder, A. alba, A. canina, A. stolonifera and A. tenuis were all found to have inductive requirement for short days but no requirement for low temperature. In order to test the effect of short-day induction, A. palustris 'Toronto' grown in 8 hour days in the greenhouse over winter was compared with material taken from the field in early spring. This comparison is shown in Table III and in Figs. 12 and 13. Since no seedheads were produced in either chilled or unchilled grass overwintered in short days, it could be stated that previous exposure to short days has an over-riding effect on both the effects of temperature pre-treatment and on the subsequent effects of photoperiod. If this is the case however, one must make the assumption that the

TABLE III. The Inductive Effect of Short-Day/Low Temperature
Pre-treatment on Creeping Bentgrass 'Toronto'

	Seedheads		Stolon Length (cms) ¹		Number Nodes ²		Internodes (cms) ¹	
	Chilled	Unchilled	Chilled	Unchilled	Chilled	Unchilled	Chilled	Unchilled
8 Hours	0.0	0.0	16.3	17.9	7.2	6.8	2.3	2.6
12 Hours	0.0	0.0	15.6	25.1	7.4	7.3	2.1	3.4
16 Hours	0.0	0.0	22.6	31.2	9.4	9.0	2.4	3.5

Data based on 10 pots (reps.).

¹Based on 5 longest stolons per pot.

²Mean number per stolon; 5 longest stolons per pot.

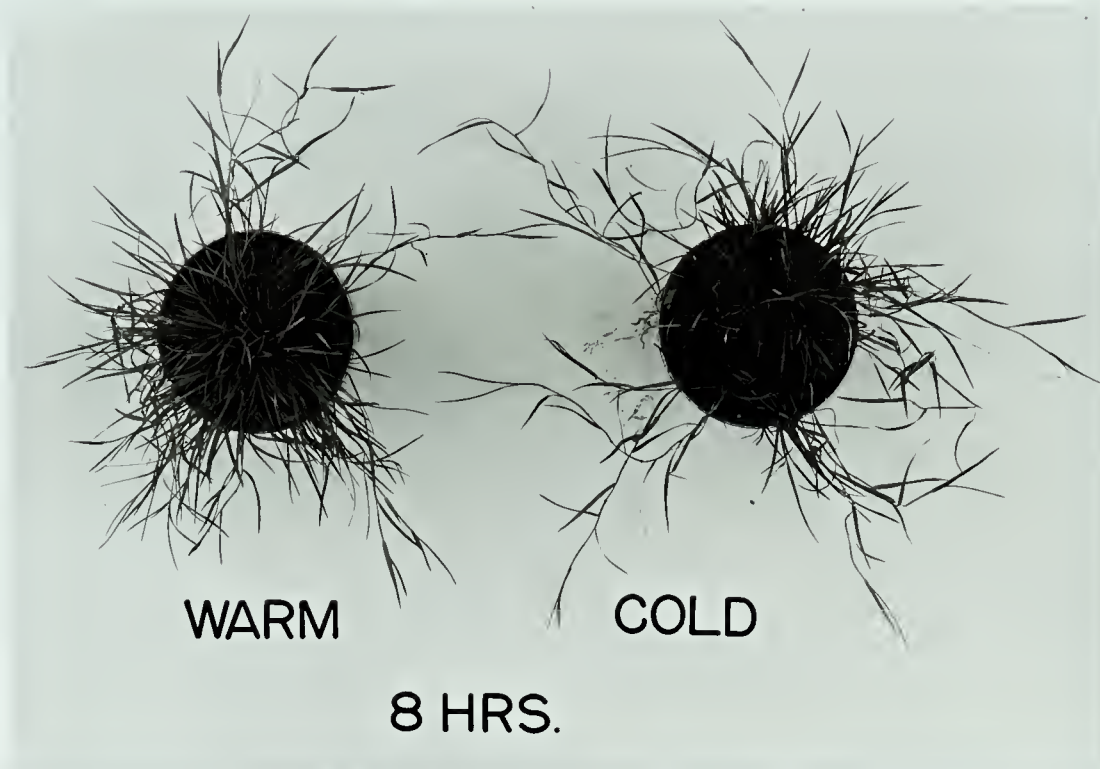


Fig. 12. Effect of inductive pretreatment (daylength + temperature) on greenhouse and field-grown Toronto creeping bentgrass.

(Chilled = Cold; Unchilled = Warm)

Photoperiod = 8 Hours

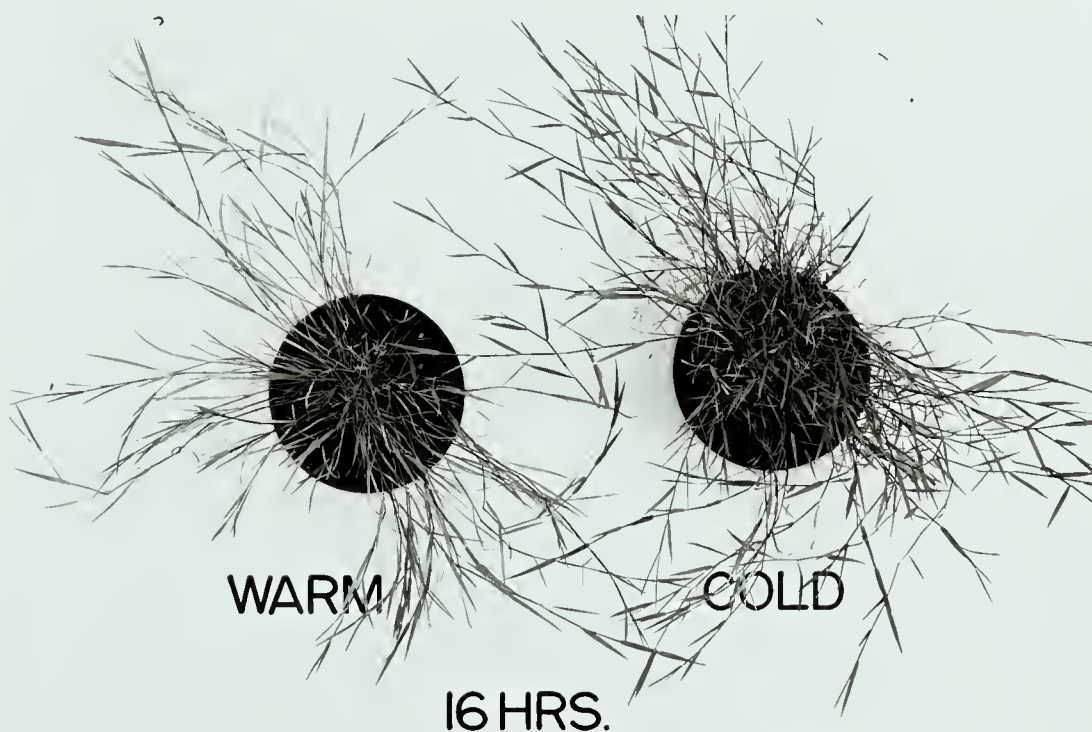


Fig. 13. Effect of inductive pretreatment (daylength + temperature) on greenhouse and field-grown Toronto creeping bentgrass.

(Chilled = Cold; Unchilled = Warm)

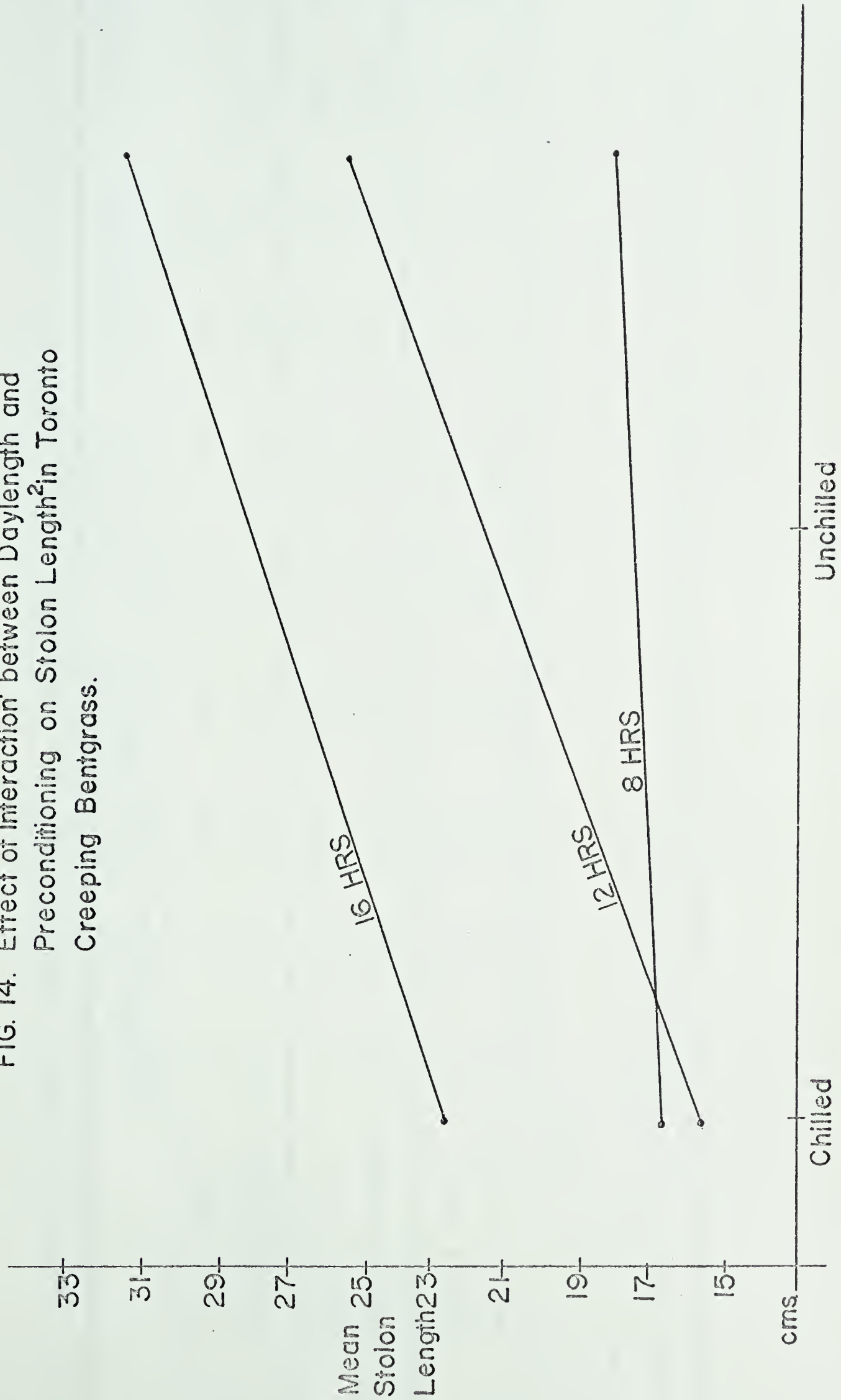
Photoperiod = 16 Hours

chilled material received sufficient short-day treatment, naturally, prior to being taken from the field and that the non-flowering condition induced had not been altered by any other factor. Because this experiment lacked precise control over daylength with chilled, field-grown material it cannot be stated that the chilled material failed to head because of short-day induction, even though it is an interesting point on which to speculate. It is of further interest to note that the variety 'Toronto' when transferred to the growth chamber in the fall (Table II), produced seedheads in 16 hour days only when it had been exposed previously to the long days of summer.

It is evident from Table III that short-day induction had no appreciable effect on the behaviour of vegetative characteristics, since unchilled material continued to show the same vegetative increase over that of chilled material that will be noted in Table IV. It may be noted from Fig. 14 and from the data in Table III, however, that significant interaction between daylength and preconditioning on stolon length is due primarily to data for 8 and 12 hour photoperiods.

To compare representative strains of the vegetative and heading types of A. palustris, material from the cultivars 'Toronto' and 'Pennecross' was selected for study. Data comparing stolon and heading behaviour in the two strains are presented in Table IV. With respect to the production of seedheads, these data appear to support the earlier findings that chilling or exposure to the low temperatures of winter is necessary for flowering to occur in these strains of

FIG. 14. Effect of Interaction¹ between Daylength and Preconditioning on Stolon Length² in Toronto Creeping Bentgrass.



Preconditioning

¹ F, Interaction = 10.4**

² Data based on 5 longest stolons per pot, 10 pots

TABLE IV. The Response of Chilled and Unchilled Toronto and Penncross Creeping Bentgrass to Variation in Daylength

	Seedheads ¹		Stolon Length (cms)		Number Nodes ²		Internodes (cms)		Stolons ^{1,3}		
	Toronto Penncross		Toronto Penncross		Toronto Penncross		Toronto Penncross		Toronto Penncross		
8 Hrs.	Chilled (Cold)	2.3	3.0	12.5	3.5	5.6	1.6	2.2	2.3	7.4	2.6
	Unchilled (Warm)	0.0	0.0	22.0	5.7	6.7	2.5	3.3	2.3	13.2	5.8
16 Hrs.	Chilled (Cold)	3.0	16.8	23.8	13.6	7.6	4.9	3.0	2.8	28.0	11.6
	Unchilled (Warm)	0.0	0.0	32.6	19.6	9.1	6.5	3.6	3.0	35.2	31.2
24 Hrs.	Chilled (Cold)	6.1	21.6	20.7	16.0	9.0	6.0	2.3	2.7	37.6	19.4
	Unchilled (Warm)	0.0	0.0	29.0	20.6	10.0	8.0	2.9	2.6	37.8	36.6

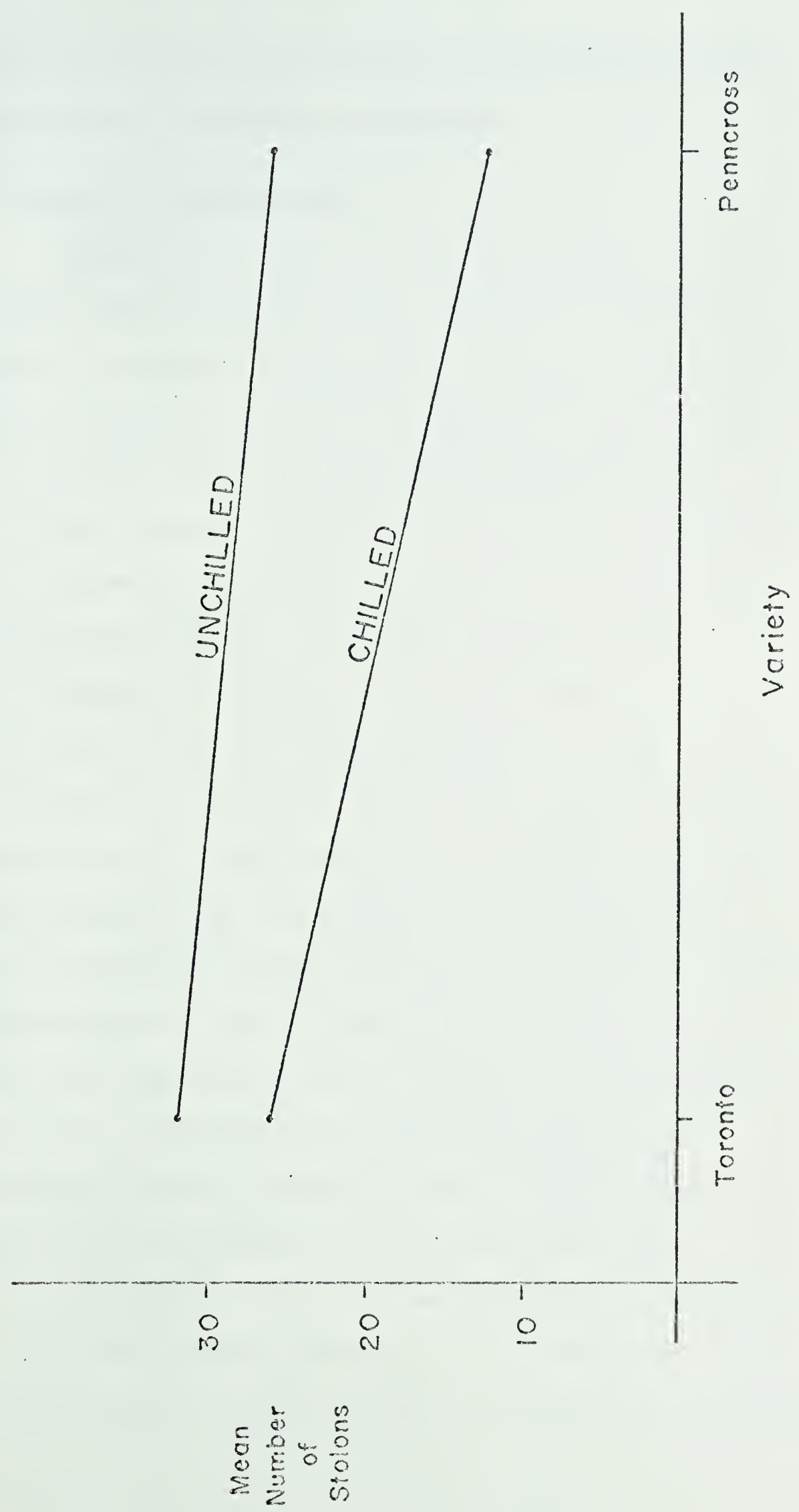
Data based on 5 pots (reps.).

¹Mean number per pot.²Mean number per stolon; 5 longest stolons per pot.³F = 142.0** (daylength); F = 46.1** (temperature preconditioning); F = 5.2* (daylength x temperature preconditioning).

creeping bentgrass. It may also be seen from these data that longer daylengths, i.e., 16 hours or more, resulted in substantially greater numbers of seedheads particularly in the heading type 'Penncross'. These data also tend to support the contention that the variety 'Penncross' belongs to the group which flowers with relative ease as distinct from the vegetative type 'Toronto'.

A significant difference in number of stolons between the two strains will be seen in Table IV. There was also high significance in the data for stolon numbers due to daylength treatment. The effect of preconditioning on the number of stolons produced differed substantially between the two strains regardless of photoperiod. This difference was highly significant. The extent of this is shown in Fig. 15. It may be noted from this and from the data of Table IV, that unchilled 'Penncross' initiated more stolons than chilled material regardless of subsequent daylength. The interaction between daylength and preconditioning for the two varieties showed a significant effect on numbers of stolons produced.

FIG. 15 Effect of Variety¹ on Stolon² Production
in Chilled and Unchilled Material.



¹ $F = 11.9^{**}$ (preconditioning x variety).

² Mean of 5 pots.

B. The Effects of CCC (2-chloroethyl) trimethylammonium chloride
on Growth and Development of Turfgrasses

(1) On Toronto Creeping Bentgrass

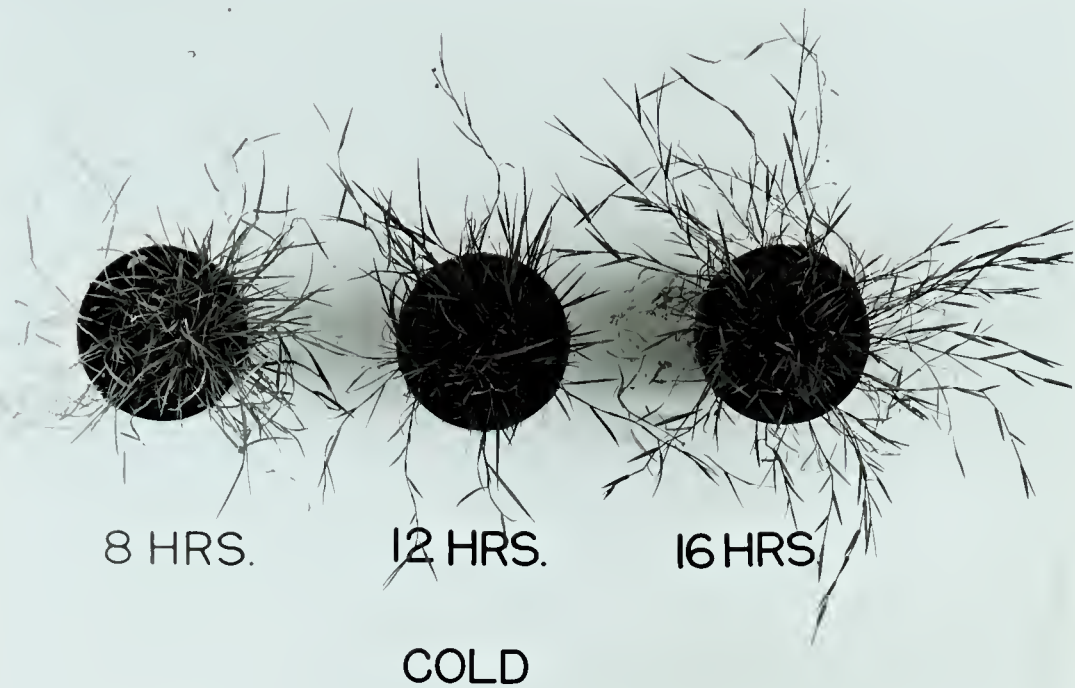
Applications of the growth retardant CCC showed no effects on seedhead production in this grass, although stolon length was substantially retarded in all daylengths particularly with material that had not been chilled (Table V). The effect of CCC on stolon length was highly significant regardless of preconditioning. Little variation was noted with respect to the number of nodes per stolon. This would indicate that the effect of CCC is manifested in the intercalary meristem rather than the shoot meristem. The effect of treatment in causing reduced stolon length but not altering the number of nodes per stolon appreciably is therefore seen in very much shorter internodes in treated plants. Differences due to CCC treatment on field and greenhouse-grown material from 8 and 16 hour photoperiods may be seen in Figs. 17 and 18 respectively. The fact that CCC had a greater effect in retarding stolon length in unchilled grass is seen in a highly significant interaction between these factors and is shown in Fig. 19. Although neither CCC treatment nor preconditioning considered separately had any significant effect on number of nodes, the interaction of these two factors indicated a statistically significant effect in this respect. This may be seen from Fig. 20 where it appears that CCC treatment, considered over all daylengths, results in greater numbers of nodes per stolon in unchilled creeping bentgrass

TABLE V. The Effect of the Growth Regulator CCC on Chilled and Unchilled Creeping Bentgrass 'Toronto'

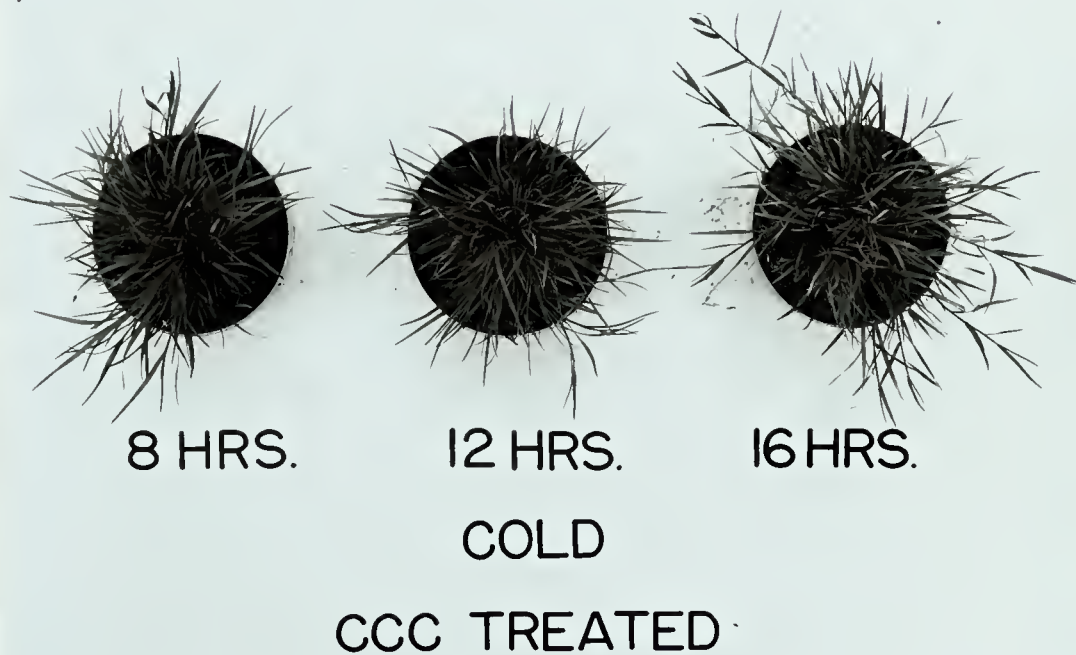
	Seedheads		Stolon Length (cms)		Number Nodes ¹		Internodes (cms)		
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	
3 Hours	Chilled (Cold)	0.0	0.0	8.1	16.6	6.2	7.2	1.3	2.3
	Unchilled (Warm)	0.0	0.0	8.1	17.9	6.6	6.8	1.2	2.6
12 Hours	Chilled (Cold)	0.0	0.0	9.8	15.6	7.6	7.4	1.3	2.1
	Unchilled (Warm)	0.0	0.0	12.8	25.1	8.2	7.3	1.6	3.4
16 Hours	Chilled (Cold)	0.0	0.0	15.8	22.6	9.4	9.4	1.7	2.4
	Unchilled (Warm)	0.0	0.0	18.1	31.2	10.0	9.0	1.8	3.5
Means				12.1**	21.5				

Data based on 10 pots (reps.).

¹Mean number per stolon; 5 longest stolons per pot.

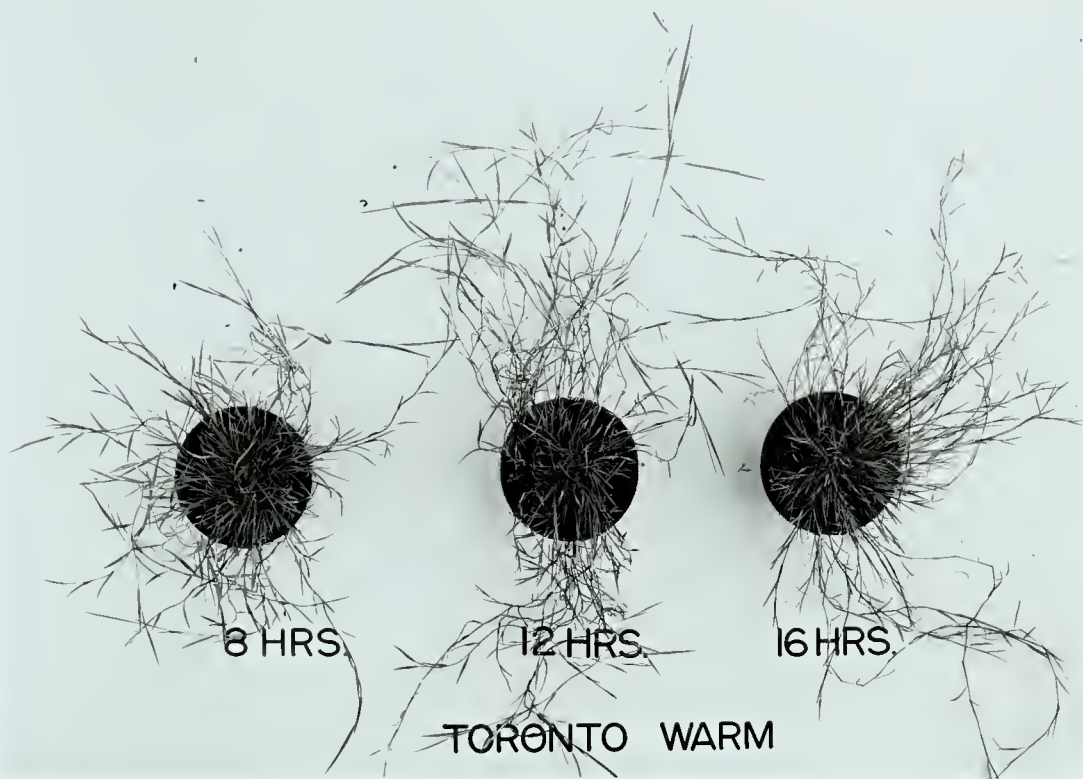


(a) Control (Cold = Chilled)

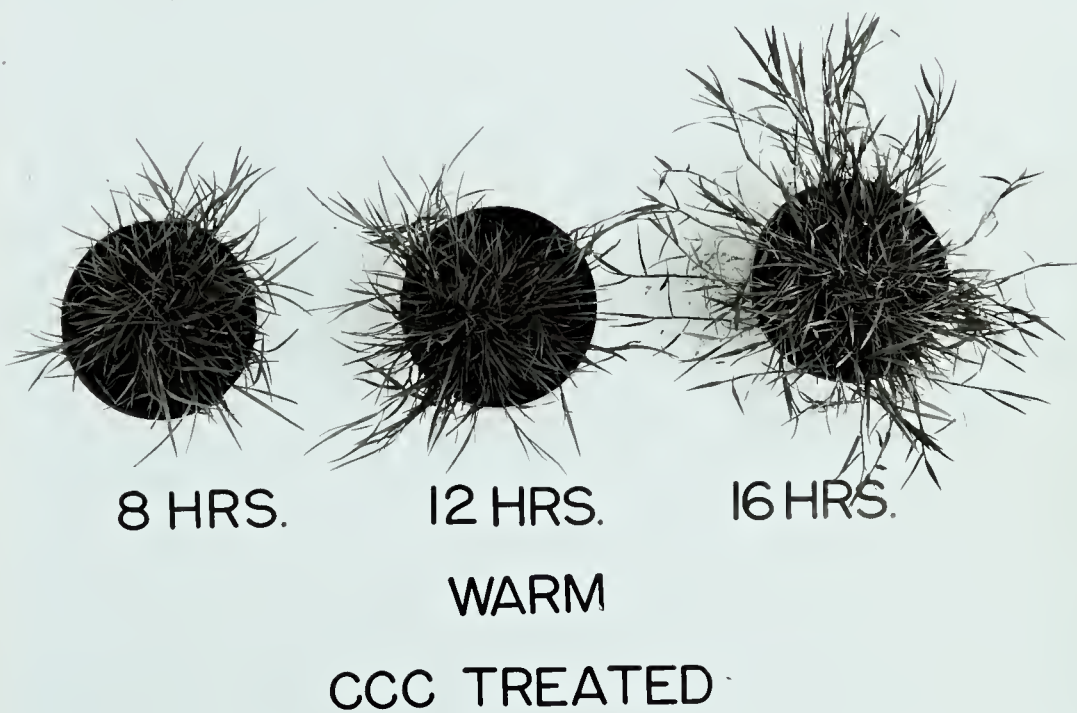


(b) CCC treated (Cold = Chilled)

Fig. 17. Effect of CCC on Toronto creeping bentgrass overwintered in the field and placed in growth chambers in early spring. (a) control plants, (b) CCC treated plants.



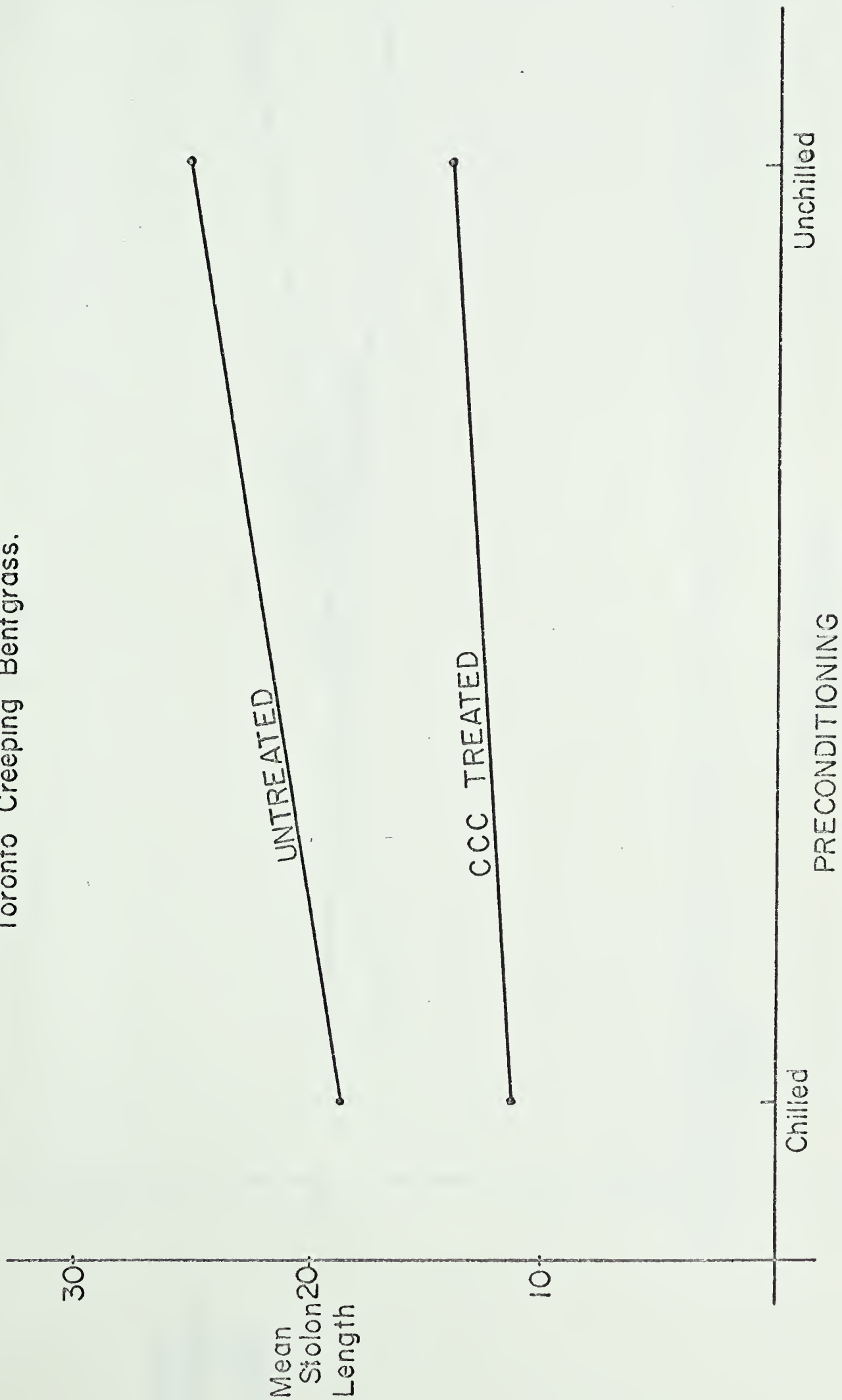
(a) Control (Warm = Unchilled)



(b) CCC treated (Warm = Unchilled)

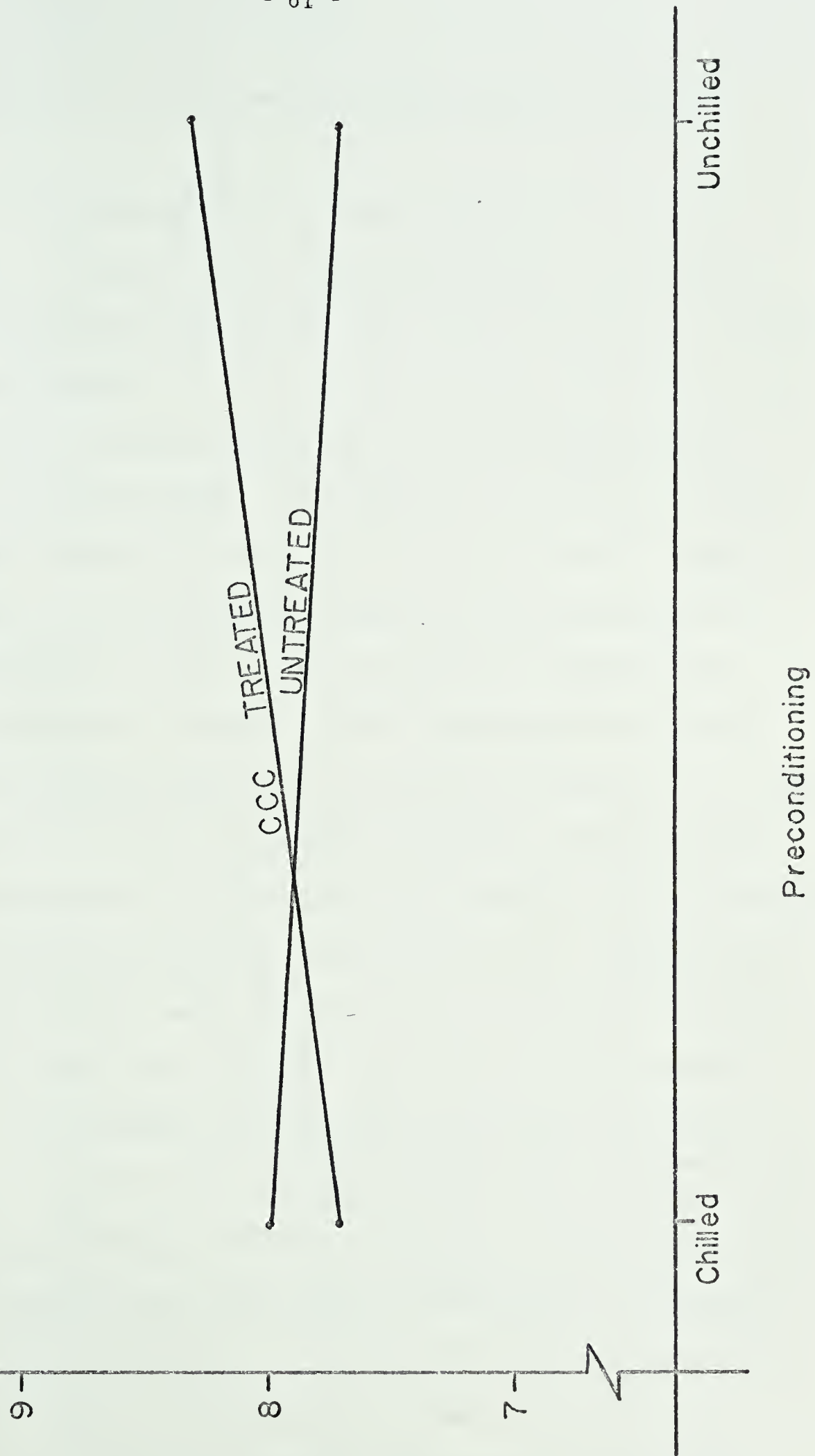
Fig. 18. Effect of CCC on Toronto creeping bentgrass overwintered in the greenhouse and placed in growth chambers in early spring. (a) Control plants, (b) CCC treated plants.

FIG.19 Differential Effect² of CCC on Stolon Length¹ in Chilled and Unchilled Toronto Creeping Bentgrass.



¹ Mean of 5 longest stolons from 10 pots
² F, interaction CCC Preconditioning = 19.8**

FIG 20 Effect of Interaction¹ of CCC Treatment and Preconditioning on Number of Nodes per Stolon² in Toronto Creeping Bentgrass.



¹F, interaction = 4.2 *
²Data from 5 longest stolons per pot 10 pots.

and fewer nodes in chilled material when compared to untreated plants.

The action of CCC as a check on growth of the strain 'Toronto' in longer (20 and 24 hour) daylengths was investigated on the theory that treatment with this chemical under these conditions might forced heading. It will be noted, however, (Table VI) that no seedheads were produced in either 20 or 24 hour photoperiods by treated or untreated grass. The inability of the 'Toronto' strain to produce seedheads in most instances indicates that the proper conditions for the developmental sequence proposed by Cooper and Calder (20) have not been met in the experiments reported here. Following inductive treatment, specific requirements for light and temperature during initiation and subsequent development, must be met for flowering to occur. For example, temperature during the dark period may have been an inhibiting factor, although this was maintained at 13° C which is not usually considered too high. Although shoot dissections were not made, no blind shoots or improperly developed heads were noted in the 'Toronto' strain in any of the conditions tested. It is possible that although the materials were induced to flower, a temperature effect at the stage of floral initiation could have prevented heading. Too high a temperature during this stage could prevent initiation even though daylength conditions were conducive. In any event, discrepancies in seedhead production in this strain even under identical growing conditions, but in different years, support the contention that heading requirements for this variety must be complex and precise.

TABLE VI. Effect of CCC on Field-grown Creeping Bentgrass 'Toronto'
Placed in the Growth Chamber Following the Growing Season (October 15)

	Seedheads ¹		Stolon Length (cms)		Number Nodes ²		Internodes (cms)		Stolons ¹	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
20 Hours	0.0	0.0	30.2	34.4	18.2	23.3	1.7	1.5	23.3	43.2
24 Hours	0.0	0.0	20.4	28.6	15.8	17.1	1.3	1.7	40.2	49.1
Means			25.3**	31.5						

Data based on 10 pots (reps.).

¹Mean number per pot.

²Mean number per stolon; 5 longest stolons per pot.

The effect of CCC on field-grown 'Toronto' taken October 15th after a summer's growth was again highly significant with respect to stolon length (Table VI). These and other morphological differences due to CCC treatment may be seen in Figs. 21 and 22. In addition to retarding stolon length in creeping bentgrass, CCC-treated material showed a greater number of erect shoots which may be of practical significance in putting green management since matting and other undesirable growth characteristics are often associated with basally decumbent culms.

During the course of the experimental work with this grass it was noted that stem growth was more erect in field-grown material early in the spring than it was at the end of the growing season. This may or may not be associated with winter exposure, but it appears from Figs. 21 and 22 that CCC treatment may have somehow replaced low temperature exposure since these grasses were taken after a summer's growth in the field.

The data of Table VI also show differences in numbers of stolons as a result of treatment and these are consistent in both daylengths. Variation in the data due to CCC treatment was found to be highly significant.

Earlier in the discussion it was noted that the strains 'Toronto' and 'Penncross' initiated significantly greater numbers of stolons from unchilled than chilled material. The effect of CCC on stolon initiation in fall-grown Toronto creeping bentgrass resembles the effect of low temperature exposure on this grass,



Fig. 21. Effect of CCC on Toronto creeping bentgrass taken from the field at the end of the growing season.

T_3 = Not treated; T_4 = Treated

Photoperiod = 20 Hours



Fig. 22. Effect of CCC on Toronto creeping bentgrass taken from the field at the end of the growing season.

T_1 = Not treated; T_2 = Treated

Photoperiod = 24 Hours

since significantly fewer stolons were initiated in treated plants. It was also noted here that with a decrease in the number of stolons there was a greater number of erect shoots. It is interesting to note that the interaction of CCC treatment and daylength had no significant effect on either stolon length or the number of nodes initiated.

(2) On Merion Bluegrass

The effect of CCC on dry matter yield of common Kentucky bluegrass, P. pratensis, grown in the greenhouse, was reported by Norris (51). Using foliar applications of 0, 2½, 5, 10, 20 and 40 lbs/A CCC, he observed that lower concentrations increased total dry matter yield due primarily to increased tillering and leaf production. At the higher rates of application, increased tillering and leaf production were offset by decreased stem length so that no significant increase in dry weight was obtained.

In the experiments with CCC on Merion bluegrass reported in this study, foliar applications resulted in a decrease in dry matter yield at all concentrations (see Table VII). Differences due to CCC treatment were not considered significant although due to unusual uniformity in the data a significant F value was obtained. It should be remembered, however, that the most significant effect on increased dry matter obtained by Norris was due to a 2½ lb./A treatment. Little increase was found at any of the higher rates in his work.

TABLE VII. Effect of CCC on Growth of Merion Bluegrass expressed
as Dry Matter Yield of Clippings¹

Time of Sampling	lbs/A CCC				
	0	5	10	20	40
2 weeks after treatment	3.5	3.5	3.3	3.3	3.3
4 weeks after treatment	3.7	3.7	3.5	3.5	3.4
7 weeks after treatment	4.2	4.1	4.0	4.0	3.9

¹Mean dry weight in gms; 5 pots (reps.) per treatment.

TABLE VIII. Effect of CCC on Rhizome Initiation in Merion Bluegrass

Time of Year	Gms/cu. ft. CCC			
	0	10	20	30
June - August (Experiment 1)	13.6x	-	41.2y	40.4y
September - January (Experiment 2)	8.8a	11.8ab	14.2b	23.6c

Mean number of rhizomes per container; 5 containers.

CCC applied as dry soil mix.

Treatment means not followed by the same letter are significantly different according to Duncan's new multiple range test. $F = 11.4^{**}$

All rates of CCC used in this experiment produced chlorotic mottling of the grass foliage.

In a similar experiment to determine the effect of soil drench applications of CCC on dry matter yield, increases at all concentrations of CCC were obtained after 7 weeks. These data are not reported. Since it might be expected that soil treatments of CCC would have a greater effect on underground parts, this was investigated.

One might also expect increased dry matter yield in Merion bluegrass to be associated with increased rhizome initiation. The data on numbers of rhizomes initiated due to CCC treatment is shown in Table VIII. It will be noted from the data for Experiment 1 that both 20 g/cu. ft. and 30 g/cu. ft. CCC resulted in statistically significant increases in the numbers of rhizomes initiated. The difference between the 20 g/cu. ft. and 30 g/cu. ft. treatments was not significant however.

In Experiment 2, variation in the data due to treatment was found to be highly significant.

In his observations of the effect of CCC on underground parts in Kentucky bluegrass, Norris (51) noted decreased rhizome growth which he measured as rhizome length per plant. He noted, however, that rhizome weight was slightly increased due to CCC treatment indicating shorter but thicker rhizomes. In these studies rhizome initiation was taken as the index of treatment, and this was found to be significantly increased due to CCC applications.

The growth of rhizomes in Kentucky bluegrass was found to be associated with long days (24). It may be seen from Table VIII that numbers of rhizomes initiated in this grass may also be under photoperiodic control. Significantly greater numbers of rhizomes were produced in long days (Experiment 1) than in short days (Experiment 2). It should be remembered, however, that in this experiment, grasses had been treated with CCC and the effect might be due to an interaction between the chemical and daylength.

C. Soluble Carbohydrate Studies

Green (29), in studies on several cool season turfgrasses viz. Kentucky bluegrass, creeping red fescue and creeping bentgrass variety 'Toronto', observed that sucrose was the most dominant sugar in leaf tissue. He reported, however, the presence of oligosaccharide other than sucrose as the best indicator of changes in carbohydrate reserves in leaf tissue, due to the effect of nitrogen treatments. In Green's studies, fructosan (monoglucopolyfructosan) was shown to be dominant over sucrose and other sugars in lower leaves and stubble in these grasses.

In these studies, experiments were conducted to detect trends in levels of soluble carbohydrates prior to changes in growth habit and to compare the levels in chilled and unchilled creeping bentgrass from short and long photoperiods. Although the strain 'Toronto' failed to flower under these conditions in this experiment, sampling was done at the beginning, middle and end of the growing

period during which marked morphological changes took place. These changes may be seen in Figs. 2, 3, and 4.

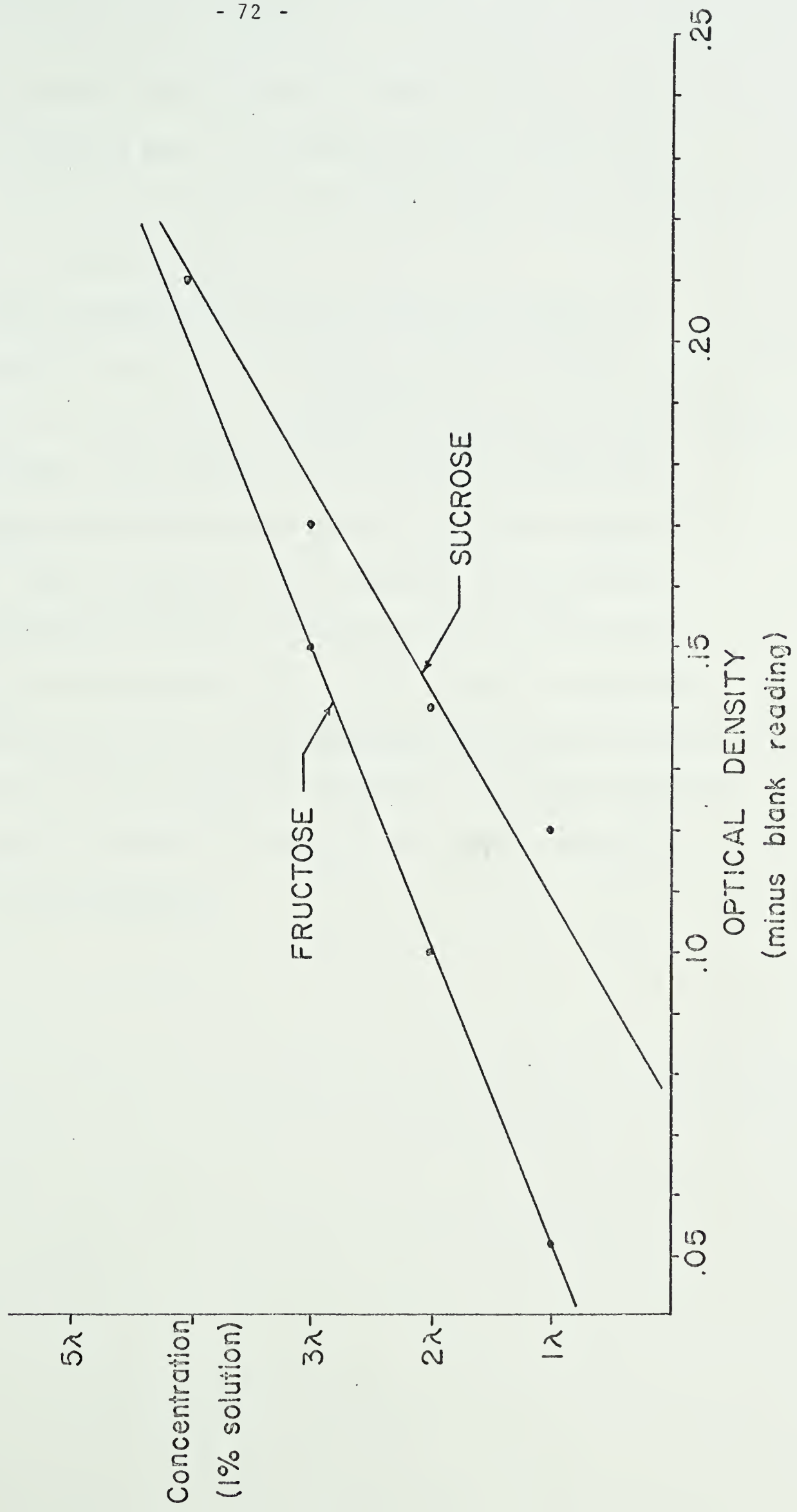
The results of determinations of carbohydrate fractions done on a semi-quantitative basis, are shown in Table IX. Extracts of tissue samples were compared on a scale constructed from known concentrations of sucrose and fructose, the standard curves of which closely approximated a straight-line relationship, and are shown in Fig. 23.

Sucrose was observed to be the dominant alcohol-soluble sugar. It gradually increased during short days in tissues from both chilled and unchilled plants. In 16 hour photoperiods sucrose was detectable in larger amounts but the levels fell in both chilled and unchilled grasses as stolons elongated and the tissues became senescent. Anthocyanin production appeared to reach a peak at the end of the sampling period.

Archbold (4) found sucrose to be the predominant sugar in leaves of barley, the content falling with stem elongation. Other workers (46, 68) observed maximum reducing sugars in grasses to be associated with rapid vegetative growth. In these studies, glucose was detectable in unchilled grass in 16 hour photoperiods at all sampling dates. Other sugars with smaller R_F values than sucrose appeared on the chromatogram but were assumed to be oligosaccharides of sufficiently low molecular weight to be soluble in 80 percent ethanol.

Oligosaccharide, other than sucrose, was noted in tissue from unchilled grass grown in 16 hour photoperiods. This observation was consistent over each of the three sampling periods,

FIG. 23 Standard Curves for Sucrose and Fructose
from Densitometer Readings.



during which stolons were in evidence. This was also observed in tissue from unchilled grass that had been grown in 8 hour days. However, in this case it was noted only after stolons had elongated, on the last two sampling dates.

The polysaccharide fructosan tended to accumulate in Toronto creeping bentgrass only toward the end of the growing period. This was particularly true of material exposed to the longer daylengths. Such results tend to support the contention that fructosan accumulation occurs primarily in stems, culms and leaf bases. This is apparent from a comparison of the data from chilled and unchilled grasses that had been grown in 8 hour days, Table IX. As observed earlier, stolons had already been initiated in unchilled grass by the time the experiment started and it will be noticed that fructosan appeared initially, only in this material. Fructosan was not recorded in chilled tissues until 31 days after the start of the experiment.

TABLE IX. Changes in the Levels of Soluble Carbohydrates in Chilled and Unchilled Creeping Bentgrass 'Toronto' During Exposure to Short and Long Daylengths

Sampling Time	Relative Densities			
	8 Hours		16 Hours	
	Chilled	Unchilled	Chilled	Unchilled
(i) Sucrose				
After 18 days in growth chamber	--1	-1	1+	1
After 31 days in growth chamber	-1	-1	2	2
After 50 days in growth chamber	1	1	1	1+
(ii) Fructosan				
After 18 days in growth chamber	nil	1++	nil	2
After 31 days in growth chamber	trace	2	-4	4
After 50 days in growth chamber	2	2	5	-4

Note: Samples in 8 hour photoperiods contained an average of 85.1% moisture by weight. In 16 hours, 73.5% moisture by weight.

Samples taken in triplicate.

Based on a comparative scale from --1 (least) to 5++ (most).

Trace detectable only under ultraviolet light.

SUMMARY AND CONCLUSIONS

Photoperiod was shown to have noticeable effects on the behaviour of five strains of creeping bentgrass. Two of these, 'Toronto' and 'Northland' showed vigorous vegetative growth over the range of daylengths studied. 'Congressional', 'Pennncross', and 'Waukanda' on the other hand, produced no stolons during short days but were able to develop seedheads under these conditions. In long days, however, their ability to produce both seedheads and stolons was greatly enhanced.

Stolon production varied greatly with variety; 'Toronto' and 'Northland' were shown to be much better than any of the others even in short days. 'Congressional' and 'Waukanda', on the other hand, seemed weakly vegetative even in long days. Such behavioural responses tend to explain why 'Toronto' and 'Northland' are regarded as the two "earliest" bentgrasses for use on putting greens, and why golf course superintendents look upon 'Congressional' and 'Waukanda' as the two strains which require the least intensive management.

While the photoperiod effect in creeping bentgrass generally is a response to long days and is expressed both in seedhead and stolon production, this is apparently not the only factor involved. The inductive effect of temperature, was also shown to be intimately associated with both seedhead and stolon production, in most of the strains studied. While low temperature appeared to be obligate for the formation of seedheads in all strains, it was not so, relative to stolon production. Experiments

conducted with the strains 'Toronto' and 'Penncross', indicated that stolon production was indeed greater in materials that had not been exposed to low temperatures. The same was true for stolon length; longer stolons being produced by unchilled material.

It was apparent in these studies, that the inductive effect of low temperature on the appearance of seedheads, operated in the same direction as that of increasing the length of day. In other words, the effects of low temperature and long days were cumulative. This is well illustrated in Table II, where it may be noted that the inductive effect of low temperature on seedhead production is greatly diminished by the end of the growing season. There were no seedheads produced by grass taken from the field at the end of summer, when it was exposed to short days. However, when the daylength was increased to 16 hours, there was marked response, though it was far less than that exhibited by material which had been taken from the field at the end of winter. This also suggests that the magnitude of the effect of low temperature preconditioning normally diminishes with time.

Although CCC was highly effective in controlling growth in the region of the intercalary meristem, it did not mask entirely the effect of temperature and daylength on stolon production. At the same time its growth regulating effect failed to change the plant material from a vegetative to a reproductive state.

Contrary to that which has been reported elsewhere, the growth regulator CCC did not increase dry weight yield of Marion

bluegrass. It is interesting to note however, that the chemical did have a marked effect on rhizome initiation in plants taken during the active growing season, and again immediately following it. While the response of CCC was consistent in both experiments, the greater number of rhizomes produced by summer-grown material was to be expected and, since the only variable between the two was length of day, it must be concluded that rhizome initiation in this grass is conditioned by long days.

From studies of soluble carbohydrates in the strain 'Toronto', it would appear that the accumulation of sucrose is associated only with increased length of day. Sucrose tends to reach a peak during long days when stolons have developed but after this, the levels drop. Although slightly higher levels of sucrose were noted initially, in chilled material growing in long days, the concentrations from both chilled and unchilled grass remained almost identical over the remainder of the growing period. Higher levels of sucrose might normally be expected in grasses having a greater ratio of leaf to stem tissue. Since unchilled material had already been producing stolons prior to placement in the growth chamber, one might expect to find lower levels of sucrose in this grass than in the chilled material.

Greater differences in soluble carbohydrates were noted in the polysaccharides of creeping bentgrass tissue. The polysaccharide studied was identified as a fructosan. Early in the growing period, higher levels of fructosan were recorded in unchilled

material, and this trend continued regardless of the daylength to which the grass was exposed. Since fructosan accumulates primarily in both stem and culm tissues, the occurrence of this material is undoubtedly related to the observation that unchilled plants had already initiated stolons prior to photoperiod treatments. This is substantiated by the fact that the marked initial difference in fructosan levels tended to even out in each daylength, as the grass became more similar in appearance.

The accumulation of fructosan in creeping bentgrass has been associated with stolon elongation and while it has been noted here that longer stolons are produced by unchilled materials, it is surprising to find that in long photoperiods chilled grass accumulates the greater amount of fructosan. A possible explanation for this might be related to the ability of only chilled material to produce seedheads. Other workers (4, 46), have observed peak concentrations of fructosan at the time of heading in cereals and in other cool-season grasses. With respect to the present study, it is obvious that the combination of chilling and long days had favoured fructosan accumulation, however, since the grass failed to head, one would have to assume that the fructosan levels had not reached threshold values associated with the phenomenon. This might explain why 'Toronto' creeping bentgrass does not flower readily.

LITERATURE CITED

1. Albrecht, H.R. Strain differences in tolerance to 2,4-D in creeping bent grasses. J. Amer. Soc. Agron. 39: 163-165. 1947.
2. Allard, H.G., and Morgan W. Evans. Growth and flowering of some tame and wild grasses in response to different photoperiods. J. Agr. Res. 62: 193-228. 1941.
3. Arber, Agnes. The Gramineae. Stechert-Hafner Service Agency, Inc., New York, N.Y. Reprint 1965.
4. Archbold, H.K. Fructosans in monocotyledons. A review. New Phytol. 39: 185-219. 1940.
5. Axelrod, B. Mono- and oligosaccharides. Plant Biochemistry. Ed. James Bonner and J.E. Varner. Academic Press, New York. pp. 1054. 1965.
6. Beard, James B. Bentgrass (Agrostis spp.) varietal tolerance to ice cover injury. Agron. J. 57: 513. 1965.
7. Baldev, B., and Anton Lang. Control of flower formation by growth retardants and gibberellin in Samolus parviflorus, a long-day plant. Amer. J. Bot. 52: 408-417. 1965.
8. Borthwick, H.A. and S.B. Hendricks. Effects of radiation on growth and development. Encycl. Plant Physiol. 16: 299-330. 1961.
9. Cabler, J.F. Chemical growth substances as substitutes for high light intensities on "Tifgreen" bermudagrass. Proc. Fla. St. Hort. Soc. 76: 470-474. 6. 1963.
10. Calder, D.M. Environmental control of flowering in Dactylis glomerata L. Nature 197: 882-883. 1963.
11. Cathey, Henry M. Physiology of growth retarding chemicals. Ann. Rev. Plant Physiol. 15: 271-302. 1964.
12. Cathey, H.M. and N.W. Stuart. Comparisons of plant growth retarding activity of AMO 1618, Phosphon, and CCC. Bot. Gaz. 123: 51-57. 1961.
13. Chouard, P. Vernalization and its relations to dormancy. Ann. Rev. Plant Physiol. 11: 191-238. 1960.

14. Cooper, J.P. Studies on growth and development in Lolium. IV. Genetic control of heading responses in local populations. J. Ecol. 42: 521-556. 1954.
15. Cooper, J.P. Developmental analysis of population in the cereals and herbage grasses. II. Response to low temperature vernalization. J. Agric. Sci. 49: 361-383. 1957.
16. Cooper, J.P. The effect of temperature and photoperiod on inflorescence development in strains of timothy (Phleum spp.). J. Brit. Grassl. Soc. 13: 81-91. 1958.
17. Cooper, J.P. The use of controlled life cycles in the forage grasses and legumes. Herb. Abstr. 30: 71-79. 1960.
18. Cooper, J.P. Species and population differences in climatic response. (In) Environmental Control of Plant Growth. pp. 381-400. Ed. L.T. Evans. Academic Press, New York. 1963.
19. Cooper, J.P. and D.M. Calder. Flowering responses in herbage grasses. Welsh Plant Breed. Sta. Rept. 20-22. 1962.
20. Cooper, J.P. and D.M. Calder. The inductive requirements for flowering of some temperate grasses. J. Brit. Grassl. Soc. 19: 6-14. 1964.
21. Doorenbos, J. and S.J. Wellensiek. Photoperiodic control of floral induction. Ann. Rev. Plant Physiol. 10: 147-184. 1959.
22. Evans, L.T. The influence of environmental conditions on inflorescence development in some long day grasses. New Phytol. 59: 163-174. 1960.
23. Evans, L.T. Reproduction. (In) Grasses and Grasslands. Ed. C. Barnard, The MacMillan Company of Canada Limited, Toronto. 1964.
24. Evans, Morgan W. and James W. Watkins. The growth of Kentucky bluegrass and of Canada bluegrass in late spring and in autumn as affected by the length of day. J. Amer. Soc. Agron. 31: 767-774. 1939.
25. Fletcher, R.A., and A.J. Renney. Effects of CCC and AMAB on bentgrass, bean and tomatoes. Proc. Nat. Weed Comm., Western Section. p. 120. 1960.
26. Gardner, F.P. and W.E. Loomis. Floral induction and development in orchard grass. Pl. Physiol. 28: 201-217. 1953.

27. Garner, W.W. and H.A. Allard. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. J. Agr. Res. 18: 553-606. 1920.
28. Goodwin, T.W. Chemistry and Biochemistry of Plant Pigments. Academic Press, New York. pp. 583. 1965.
29. Green, David G. Seasonal responses of soluble carbohydrates in the leaves of four cool-season grasses to five nitrogen treatments. M.Sc. thesis. Michigan State University. 1961.
30. Guttridge, G.C. The effect of (2-chloroethyl) trimethylammonium chloride on the growth and runnering of strawberry plants. Hort. Res. 3: 79-83. 1964.
31. Hansen, R.C., et. al. A review of the carbohydrate constituents of roughages. Univ. of Ill. Agric. Exp. Sta. Bull. 634. pp. 47. 1958.
32. Harborne, J.B. Flavonoid pigments. (In) Plant Biochemistry. Ed. James Bonner and J.E. Varner. Academic Press, New York pp. 1054. 1965.
33. Hitchcock, A.S. Manual of the Grasses of the United States. Second edition revised by Agnes Chase. U.S.D.A. Misc. Publ. 200. 1951.
34. Holt, E.C. and R.L. Davis. Differential responses of Arlington and Norbeck bent grasses to kinds and rates of fertilizers. J. Amer. Soc. Agron. 40: 282-284. 1948.
35. Holt, E.C., and K.T. Payne. Variation in spreading rate and growth characteristics of Creeping Bentgrass seedlings. Agron. J. 44: 88-90. 1952.
36. Joiner, J.N. and R.T. Poole. Variable photoperiod and CCC effects on growth and flowering of Gardenia jasminoides 'Veitchii'. Proc. Fla. St. Hort. Soc. 75: 449-451. 1963.
37. Joiner, J.N. and T.J. Sheehan. Effects of photoperiod, propagation date and dwarfing compounds on growth and flowering of poinsettias. Proc. Fla. St. Hort. Soc. 75: 451-456. 1963.
38. Juska, Felix V. Shade tolerance of bentgrasses. Golf Course Reporter. 3: 28-34. 1963.

39. Juska, F.V., and A.A. Hanson. Effects of growth retardants on Kentucky bluegrass turf. Golf Course Reporter. June, 1964.
40. Ketellapper, H.J. Growth and development in Phalaris. I. Vernalization response in geographic strains of P. tuberosa L. Ecol. 41: 298-305. 1960.
41. Laidlaw, R.A. and Clare B. Wylam. Analytical studies on the carbohydrates of grasses and clovers. II. The preparation of grass samples for analysis. J. Sci. Fd. Agric. 3: 394-500. 1952.
42. Lang, Anton. Physiology of flowering. Ann. Rev. Plant Physiol. 3: 265-306. 1952.
43. Libbert, von E., and I. Urban. Förderung der stecklingsbewurzelung bei windepflanzen durch das 'antigiberellin' 2-chlorathyltrimethylammonium chlorid. Die Naturwissenschaften 5: 92-93. 1964.
44. Lindstrom, R.S. and N.E. Tolbert. (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. IV. Effect on chrysanthemums and poinsettias. Quart. Bull. Michigan Agr. Exp. St. 42: 917-928. 1960.
45. Lockhart, James A. Mechanism of the photoperiodic process in higher plants. Encycl. Plant Physiol. 16: 390-438. 1961.
46. Lopatecki, L.E. The nature and origin of wheat stem glucofructans. Ph.D. thesis. University of Alberta. 1958.
47. May, L.H. The utilization of carbohydrate reserves in pasture plants after defoliation. Herb. Abstr. 30: 239-245. 1960.
48. Murneek, A.E. and R.D. Whyte. Vernalization and Photoperiodism. Lotsya, A., Ed., Chronica Botanica, Waltham, Mass. 193pp. 1948.
49. Naylor, Aubrey W. The photoperiodic control of plant behaviour. Encycl. Plant Physiol. 16: 331-389. 1961.
50. Neumüller, Gunnar. Pentosans and hexosans (excluding cellulose) (In) Encyclopedia of Plant Physiology. Ed. W. Rukland, Springer-Verlag, Berlin. pp. 1444. 1958.
51. Norris, R.F. Some morphological and physiological effects of (2-chloroethyl) trimethylammonium chloride (CCC) on certain species of gramineae. Ph.D. thesis. University of Alberta. 1964.

52. North, H.F.A. and T.E. Odland. The relative seed yields in different species and varieties of bent grass. J. Amer. Soc. Agron. 27: 374-383. 1935.
53. Peterson, M.L., Cooper, J.P. and L.F. Bendixen. Thermal and photoperiodic induction of flowering in darnel (Lolium temulentum). Crop Sci. 1: 17-20. 1961.
54. Peterson, Maurice L. and W.E. Loomis. Effects of photoperiod and temperature on growth and flowering of Kentucky bluegrass. Pl. Physiol. 24: 31-43. 1949.
55. Purvis, O.N. The physiological analysis of vernalization. Encycl. Plant Physiol. 16: 76-122. 1961.
56. Roberts, Eliot C. and A.E. Cott. Comparative value of bentgrass strains for putting greens. Professional Turfgrass Series. Iowa State Univ. Coop. Ext. Ser. 1965.
57. Roberts, R.N. and B.E. Struckmeyer. The effects of temperature and other environmental factors upon the photoperiodic responses of some of the higher plants. J. Agric. Res. 56: 633-677. 1938.
58. Salisbury, Frank B. Photoperiodism and the flowering process. Ann. Rev. Plant Physiol. 12: 293-326. 1961.
59. Salisbury, Frank B. The Flowering Process. The Macmillan Company, New York. 1963.
60. Smith, D.C. The breeder's ways and means. Grass - Yearbook of Agriculture. U.S.D.A., Washington. 1948.
61. Steele, C.C. An Introduction to Plant Biochemistry. G. Bell and Sons, London. pp. 346. 1949.
62. Stoddart, J.L. An assessment of (2-chloroethyl) trimethylammonium chloride as a potential aid to grass-seed production. J. Brit. Grassl. Soc. 19: 373-375. 1964.
63. Stuart, N.W. Initiation of flower buds in Rhododendron after application of growth retardants. Science. 134: 50-52. 1961.
64. Stuckey, I.H., and W.G. Banfield. The morphological variations and the occurrence of aneuploids in some species of Agrostis in Rhode Island. Amer. J. Bot. 33: 185-190. 1946.
65. Sullivan, J.T. and V.G. Sprague. The effect of temperature on the growth and composition of the stubble and roots of perennial ryegrass. Plant Physiol. 24: 706-718. 1949.

66. Technical Manual, A. Experimental Plant growth regulant CCC (2-chloroethyl) trimethylammonium chloride. Am. Cyanamid Co., Stanford, Conn., U.S.A. February, 1961.
67. Tolbert, N.E. (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. II. Effect on growth of wheat. Plant Physiol. 35: 380-385. 1960.
68. Troughton, A. The underground organs of herbage grasses. Commonwealth Bureau of Pastures and Field Crops. Bull. 44. 1957.
69. Watkins, James M. The growth habits and chemical composition of brome grass, Bromus inermis Leyss. as affected by different environmental conditions. J. Amer. Soc. Agron. 32: 527-538. 1940.
70. Weinmann, Hans. Carbohydrate reserves in grasses. Proc. 6th Internal. Grassl. Congr. 1: 655-660. 1952.
71. Zeevaart, J.A.D. Effects of the growth retardant CCC on floral initiation and growth in Pharbitis nil. Plant Physiol. 39: 402-408. 1964.
72. Zeevaart, J.A.D., and A. Lang. Suppression of floral induction in Bryophyllum daigremontianum by a growth retardant. Planta 59: 509-517. 1963.

ACKNOWLEDGEMENTS

The author wishes to sincerely thank Professor R.H. Knowles for his patience and helpful guidance throughout these investigations and for his diligent assistance in the preparation of this manuscript. Thanks are also extended to the greenhouse and horticulture division field staffs, University of Alberta, for help with the growing and maintenance of grasses, as well as to the University of Alberta for growth chamber facilities and financial aid.

Gratitude is expressed to Mrs. Maureen Shargool, Mr. Klaus Martin and Dr. Saul Zalik for assistance in carrying out soluble carbohydrate studies.

Dr. R.T. Hardin is thanked for his suggestions and guidance in the use of the digital computer for statistical analysis, as is Mrs. A. Kmech for the typing of this manuscript.

B29865